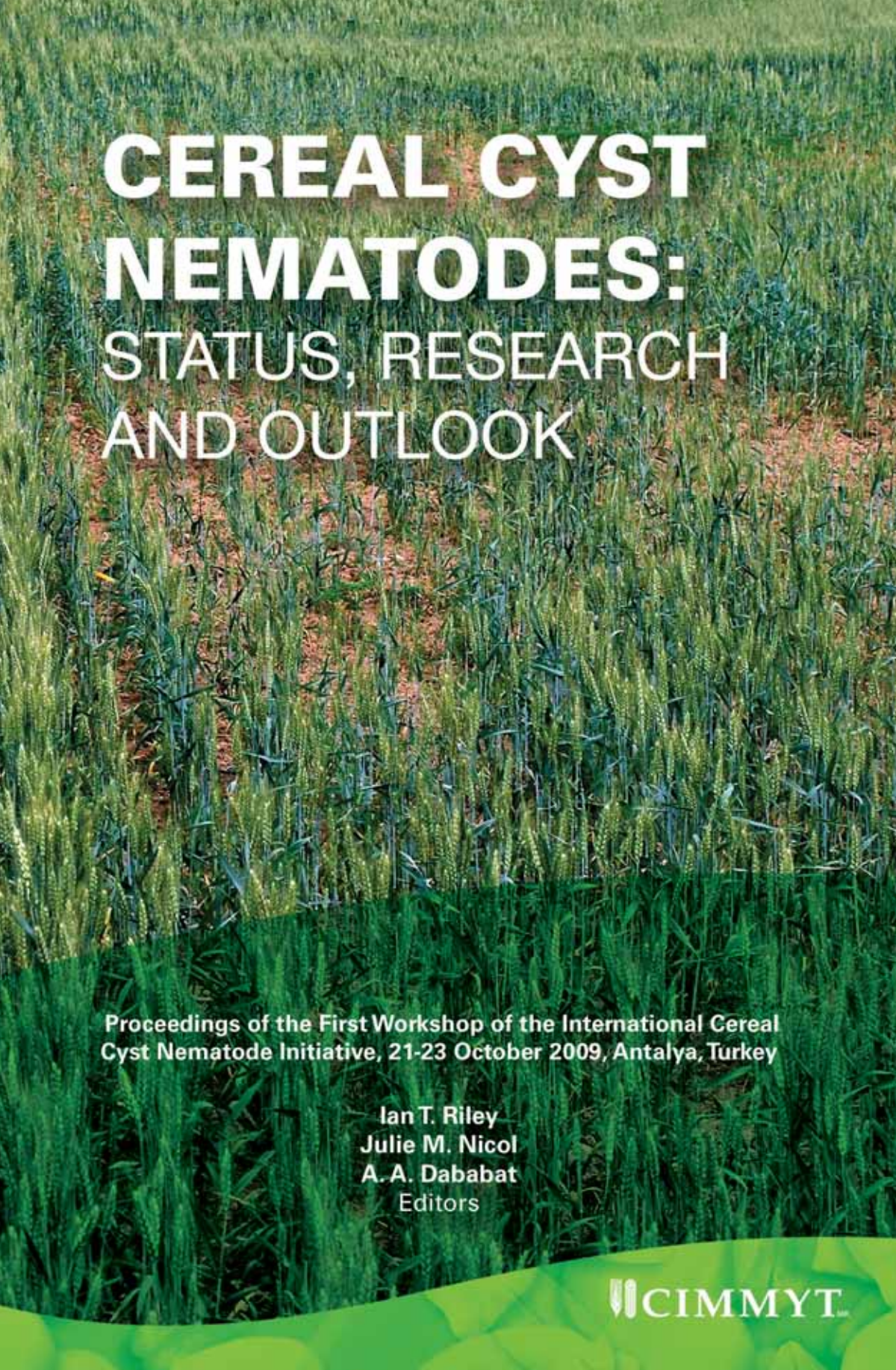

CEREAL CYST NEMATODES: STATUS, RESEARCH AND OUTLOOK

Proceedings of the First Workshop of the International Cereal Cyst Nematode Initiative, 21-23 October 2009, Antalya, Turkey

Ian T. Riley
Julie M. Nicol
A. A. Dababat
Editors

CIMMYT

CEREAL CYST NEMATODES:
STATUS, RESEARCH AND OUTLOOK

These proceedings are dedicated to the late Dr Norman Ernest Borlaug (1914-2009), the Father of the Green Revolution and the most important wheat breeder

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CIMMYT MR
International Maize and Wheat Improvement Center

CIMMYT® (www.cimmyt.org) is an internationally funded, not for-profit organisation that conducts research and training related to maize and wheat throughout the developing world. Drawing on strong science and effective partnerships, CIMMYT works to create, share and use knowledge and technology to increase food security, improve the productivity and profitability of farming systems, and sustain natural resources. CIMMYT is one of 15 Future Harvest Centres of the Consultative Group on International Agricultural Research (CGIAR) (www.cgiar.org). Financial support for CIMMYT's work comes from the members of the CGIAR, national governments, foundations, development banks and other public and private agencies.

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Abstract: The first meeting of the International Cereal Cyst Nematode Initiative, held in October 2009 in Turkey, involved over 60 scientists from wheat-growing regions in Asia, Australia, Europe, north Africa and North America. Cereal cyst nematodes (CCN) are damaging root parasites of barley, oat, wheat and related plants; the most important species being *Heterodera avenae*, *H. filipjevi* and *H. latipons*. Forty three papers in this volume cover: the history and status of CCN both globally and regionally; research on CCN morphological, genetic and ecology diversity; development and deployment of host resistance as the principal means of control, including advancements provided by molecular technology; and investigations into other types of control and opportunities for integrated management. The papers provide valuable insight into the impact of CCN and endeavours to provide sustainable management options for farmers. CCN's impact ranges from severe in resource-limited cropping systems with high pathotype diversity through to the now easily managed situation in Australia, with one pathotype and many resistant cultivars released. In many countries, unacceptable economic losses continue and international collaboration is needed to ensure that appropriate genetic resources and technology are developed, disseminated and applied where the need is greatest.

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Cover illustration: Irrigated winter wheat infested with *Heterodera avenae* with an intolerant cultivar (front), showing patches of stunted plants, and intolerant cultivar (back) growing in adjacent farmer's fields in Xuchang, Henan, China. Photograph: Ian T. Riley.

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Cereal cyst nematode (*Heterodera avenae*) in wheat. Illustration *circa* 1964 by Margaret Senior (1917-1995), a work commissioned by the New South Wales Department of Agriculture.

Foreword

Cereals constitute the world's most important source of food. Among cereals, wheat, maize and rice occupy the most eminent position in terms of production, acreage and source of nutrition, particularly in developing countries. It has been estimated that about 70% of the land cultivated for food crops is devoted to cereal crops.

The demand for wheat, based on production and stock changes, is expected to increase from 621 Mt during 2004-06 to 760 Mt in 2020[1], around 813 Mt in 2030, and more than 900 Mt in 2050[2,3]; this implies growth rates of 1.6% during 2005-20, 1.2% during 2005-30, and 0.9% over 2005-50[4]. Furthermore, the effects of global climate change, namely the increased frequencies of drought and heat stress, will impact on food security. To compensate for the additional demand for wheat, methods must be employed to minimise yield production constraints. Plant parasitic nematodes are recognised as one such constraint which has in many cases been overlooked. In wheat, cereal cyst nematode (CCN) is acknowledged globally as a biotic constraint for wheat production, particularly under rain-fed conditions and drought stress. In trials conducted in the rain-fed winter wheat areas in the Central Anatolian Plateau of Turkey losses of up to 40% on widely cultivated cultivars were observed, and there are similar yield losses reported from many other countries in the world.

Although the introduction of new wheat cultivars has boosted agricultural output, the yield potential of the new cultivars has not been fully expressed and is often far below theoretical maximum yields. This disparity between actual and theoretical yield expression can be attributed to "production constraints". Attention has therefore been focused on minimising these constraints to increase production. Although foliar wheat diseases such as rust have long been recognised as important constraints affecting wheat production, extensive research on the "weak linkages" and the unseen or underground enemy in the plant-pest system are lacking.

As most nematodes live in the soil, they represent one of the most difficult pest problems to identify, demonstrate and control. Farmers, breeders, agronomists and pest management consultants commonly underestimate their effects but it has been estimated that some 10% of the world crop production is lost as a result of plant nematode damage[5]. It is also pertinent to appreciate in the soil of the cereal systems that microscopic nematodes also interact with other plant pathogens, particularly soil

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[1]Rosegrant, M W, Paisner MS, Meijer S, Witcover J (2001) 'Global food projections to 2020: emerging trends and alternative futures.' (International Food Policy Research Institute: Washington, USA)

[2]FAO (2006) World Agriculture: Towards 2030/2050. Interim report. (Global Perspective Studies Unit, FAO: Rome, Italy)

[3]Rosegrant M, Ringler C, Msangi S, Zhu T, Sulser T, Valmonte-Santos R, Wood S (2007) Agriculture and food security in Asia: The role of agricultural research and knowledge in a changing environment. *Journal of Semi-Arid Tropics Agricultural Research* 4, 1-35.

[4]Dixon J, Braun HJ, Kosina P, Crouch J Eds (2009) 'Wheat facts and futures 2009.' (CIMMYT: Mexico).

[5]Whitehead AG (1998) 'Plant nematode control.' (CAB International: Wallingford, UK)

borne fungi, and in many cases the synergism which results in more damage than either pathogen alone.

Various methods can be employed to control nematodes, including integrated pest management systems. The most successful method to date has been the use of genetic host resistance and non-host rotational crops. Australia is an excellent example of research and application, where most farmers plant nematode resistant varieties to control CCN. It is important to stress that the most appropriate control method will be determined by the nematode species involved and the economic feasibility of implementing a management practice.

The ICARDA-CIMMYT Wheat Improvement Program (ICWIP) under the leadership of CIMMYT in collaboration with The Turkish Ministry of Agriculture and Çukurova University has been working since 2001 in a joint International collaboration on CCN. We hope this workshop where more than 22 countries are represented and over 50 scientific presentations shared will provide greater insight and awareness to the extent of the problem of CCN in wheat production systems globally. The published proceedings will serve as a baseline of current knowledge and should help foster the opportunities to establish strong collaborative interactive networks to improve wheat productivity and global food security between our National Program partners and leading Advanced Research Institutions.

Dr Hans J Braun
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A/Prof. Masum Burak
Director General, TAGEM (Turkish Ministry of Agriculture and Rural Affairs), Ankara, Turkey.

Dr Amor Yahyaoui
Coordinator, ICARDA-CIMMYT Wheat Improvement Program Coordinator, ICARDA, Aleppo, Syria.

Acknowledgments

The First International Cereal Cyst Nematode Initiative Meeting held in Turkey in 2009, coordinated by Dr Julie M. Nicol, CIMMYT could not have been successful without the assistance and support of many.

The editors sincerely thank Dr Hans J Braun, CIMMYT Global Wheat Director and Dr Vehbi Eser, Head of Field Crops Research Department, TAGEM (Turkish Ministry of Agriculture and Rural Affairs) for their leadership, support and encouragement for this joint meeting to occur.

The donors, including ICWIP (CIMMYT International), United States AID program (USAID), The Australian Centre of International Agricultural Research (ACIAR), The Australian ATSE Crawford Fund, sp-IPM (The System Wide Program of Integrated Pest Management of the CGIAR), Syngenta and TAGEM (Turkish Ministry of Agriculture and Rural Affairs), are gratefully acknowledged.

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CIMMYT (International Maize and Wheat Improvement Centre) and ICARDA (International Centre for Agricultural Research in Dryland Agriculture) are non-profit International Research Centres with the mandate to improve agriculture in developing countries as part of the Consultative Group of International Agriculture Research. CIMMYT's headquarters are in Mexico, with 15 outreach offices around the world, one of which is in Turkey. CIMMYT's mandate is improve the productivity of wheat and maize systems through sustainable management and improvement. The Turkey office works in west Asia, north Africa and Central Asia. ICARDA is based in Syria and works regionally in dryland areas on cereal, legume and animal production systems, once again improving productivity of these through sound management practices. ICWIP (ICARDA-CIMMYT Wheat Improvement Program) is the collective effort of both centres to address food security for cereals in west Asia, North Africa and Central Asia. CIMMYT is gratefully acknowledged for both scientific leadership in the research of CCN, technical input, international capacity building and financial support towards the meeting. For further information visit www.cimmyt.org and www.icarda.org.

The Systemwide Program on Integrated Pest Management of the Consultative Group on International Agricultural Research is an inter-centre initiative to develop knowledge and technologies for innovative crop protection to increase and secure the production of safe food in an environmentally and economically sound way in the developing world. The core members are CIMMYT, CIP, CIAT, ICRISAT, IFPRI, IRRI, ICARDA, WARDA, Bioversity, IITA as the host centre, and the associated centres *icipe* and AVRDC. The Program focuses on three main research areas (AIM): **A**dapting IPM to climate variability and change, **I**mproving agroecosystem resilience, and **M**anaging contaminants in food, feed, and the environment. Sp-IPM are acknowledged for their support for the joint CIMMYT-ICARDA capacity building efforts on the integrated control of soil borne pathogens, including cereal cyst nematode. More information on the sp-IPM can be found at www.spipm.cgiar.org

TAGEM is the headquarter of the Turkish national agricultural research system in Ankara. Their key objective is to develop of research strategy, determine the priorities and coordination of research programs. TAGEM is thanked for their coordination of the joint CIMMYT-Turkey collaboration on soil borne pathogens of wheat and the leadership and oversight of the joint research program on cereal cyst nematode with a number of national programs and CIMMYT.

USAID is an independent federal government agency that receives overall foreign policy guidance from the Secretary of State. The agency supports long-term and equitable economic growth by economic growth, agriculture and trade; global health; and democracy, conflict prevention and humanitarian assistance. They are thanked for their financial contributions in a joint CIMMYT-USAID linkage grant for assistance in capacity development and training. Further information can be obtained at www.usaid.gov/our_work/.

The Australian Centre of International Agricultural Research (ACIAR) is an Australian Government statutory authority that operates as part of Australia's Aid Program within the portfolio of Foreign Affairs and Trade. ACIAR contributes to the aid program objectives of advancing Australia's national interest through poverty reduction and sustainable development. They are thanked for supporting several participants to attend the meeting. More information is available at www.aciar.gov.au

The purpose of the Crawford Fund is to make more widely known the benefits that accrue both to Australia and internationally from international agricultural research and development. The Fund conducts a range of public awareness activities, arranges specialist training in Australia and abroad for developing country scientists, and conducts master classes for developing country personnel in key topics in agricultural research and development. Thanks are given for their support of participants to this meeting and also previous support for capacity building Master Classes on Soil Borne Pathogens. More information on the Crawford Fund can be found at www.crawfordfund.org.

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Disclaimer

This volume contains papers voluntarily contributed by participants of the First Workshop of the International Cereal Cyst Nematode Initiative, 21-23 October 2009, Antalya, Turkey.

The information and views expressed in these papers are entirely those of the authors. The papers have not been formally peer-reviewed and the veracity of the content has not been confirmed.

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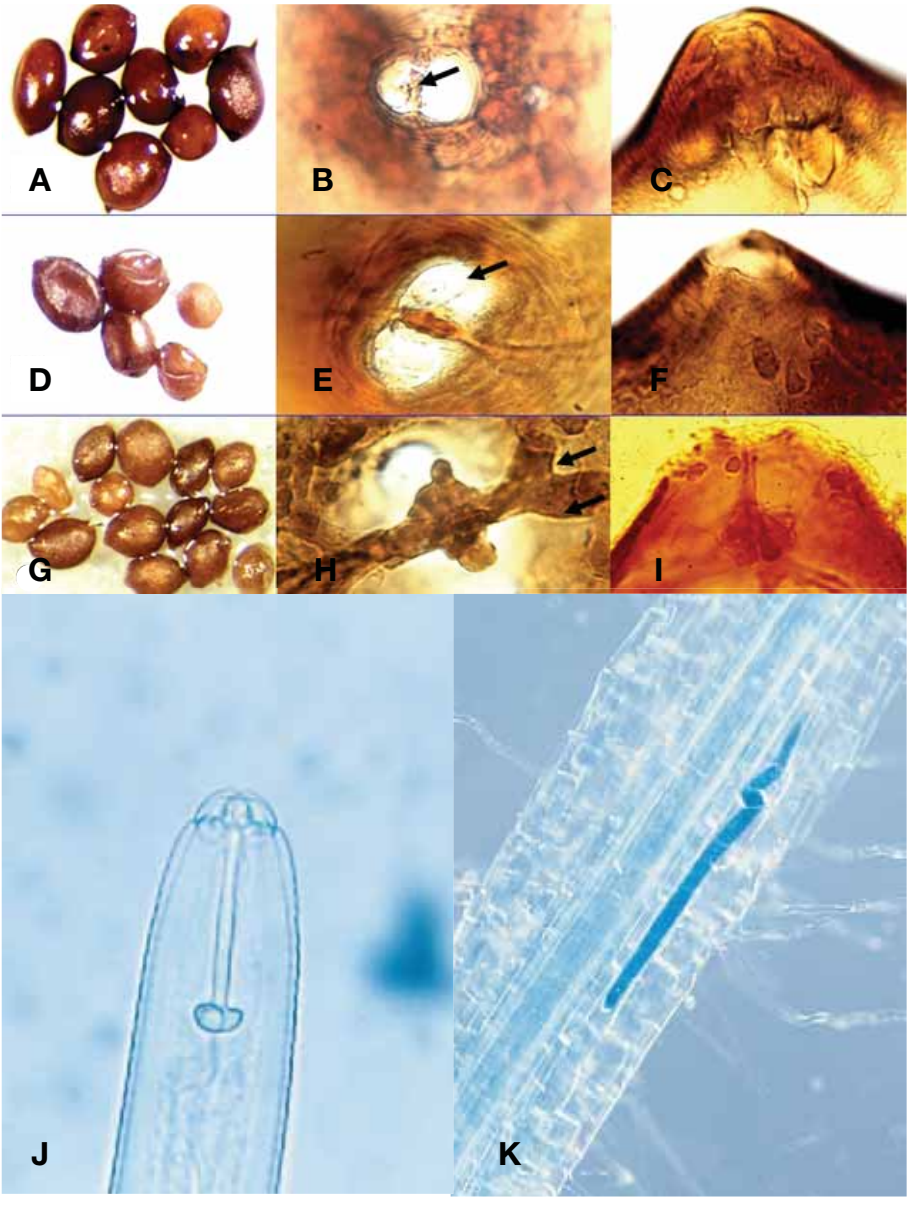

Plate 1. A-C, cysts, underbridge and vulval cone of *Heterodera avenae*; D-F, cysts, underbridge and vulval cone of *Heterodera filipjevi*; G-I, cysts, underbridge and vulval cone of *Heterodera latipons*; J, head and stylet of *H. avenae* second stage juvenile; K, second stage juvenile of *H. avenae* migrating it the cortex towards the root tip (stained). Photographs: A-I, Hussam Abiedo, Syria; J-K, Hugh Wallwork, Australia.

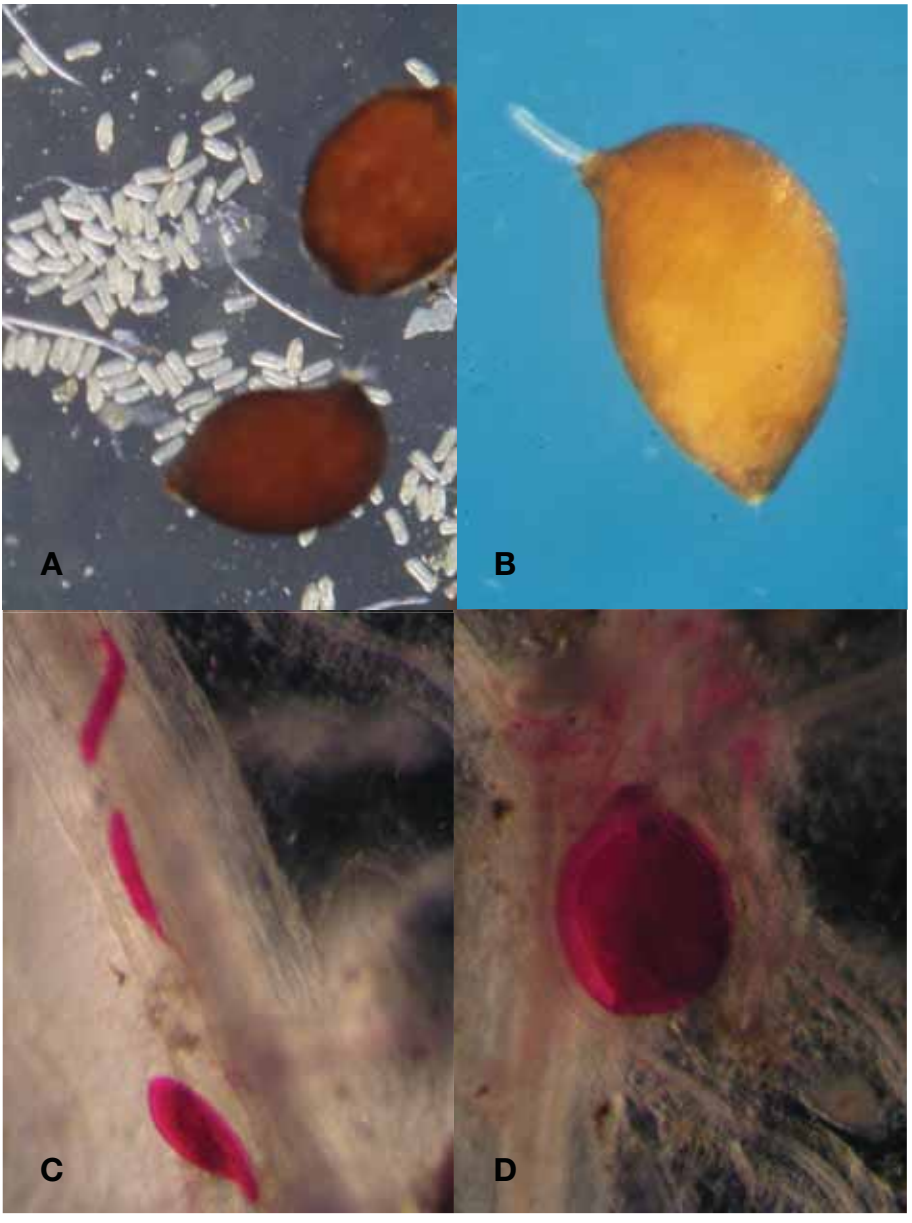

Plate 2. A, disrupted *Heterodera avenae* cyst showing eggs and second stage juveniles.; B, *Heterodera avenae* cyst with juvenile emerging through the fenestra; C, *Heterodera filipjevi* developing in wheat roots, third and fourth stage juveniles (stained); D, *H. filipjevi* immature female broken though the root epidermis (stained). Photographs: A, Najoua Namouchi-Kachouri, Tunisia; B, John Lewis, Australia; C-D, Hayriye Didem Sağlam, Turkey.

Plate 3. A, stunted wheat plants with knotted roots heavily infested with *Heterodera avenae* (left) compared uninfested plants (right), Henan, China; B, knotted wheat roots heavily infested with *H. avenae*, Henan, China; C, white female of *H. avenae*. Photographs: A-B, Honglian Li, China; C, Hugh Wallwork, Australia.

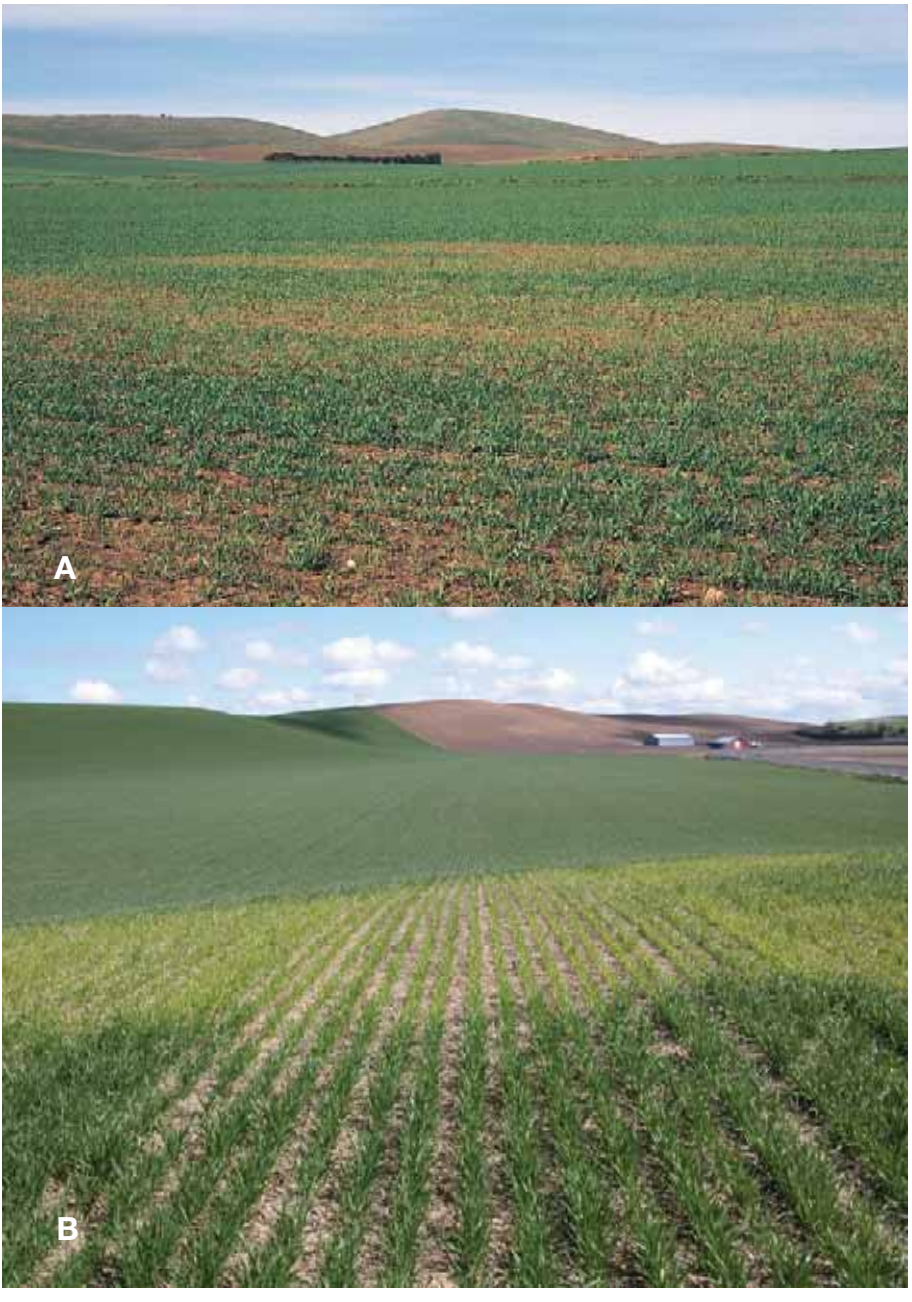

Plate 4. A, patches stunted, chlorotic plants in an oat crop infested with *Heterodera avenae* in South Australia; B, winter wheat affected by *H. avenae* combined with lower soil fertility on a hilltop near Palouse, Washington, USA. Photographs: A, Hugh Wallwork, Australia; B, Richard W. Smiley, USA.

Plate 5. A, irrigated winter wheat infested with *Heterodera avenae* with tolerant (left) and intolerant (right) cultivars growing in adjacent farmer's fields in Xuchang, Henan, China; B. durum wheat experiment in a cereal cyst nematode infested field under drought conditions in Le Kef, Tunisia with aldicarb treated (left) and untreated (right) plots (Najoua Namouchi-Kachouri). Photographs: A-B, Ian T. Riley, Australia.

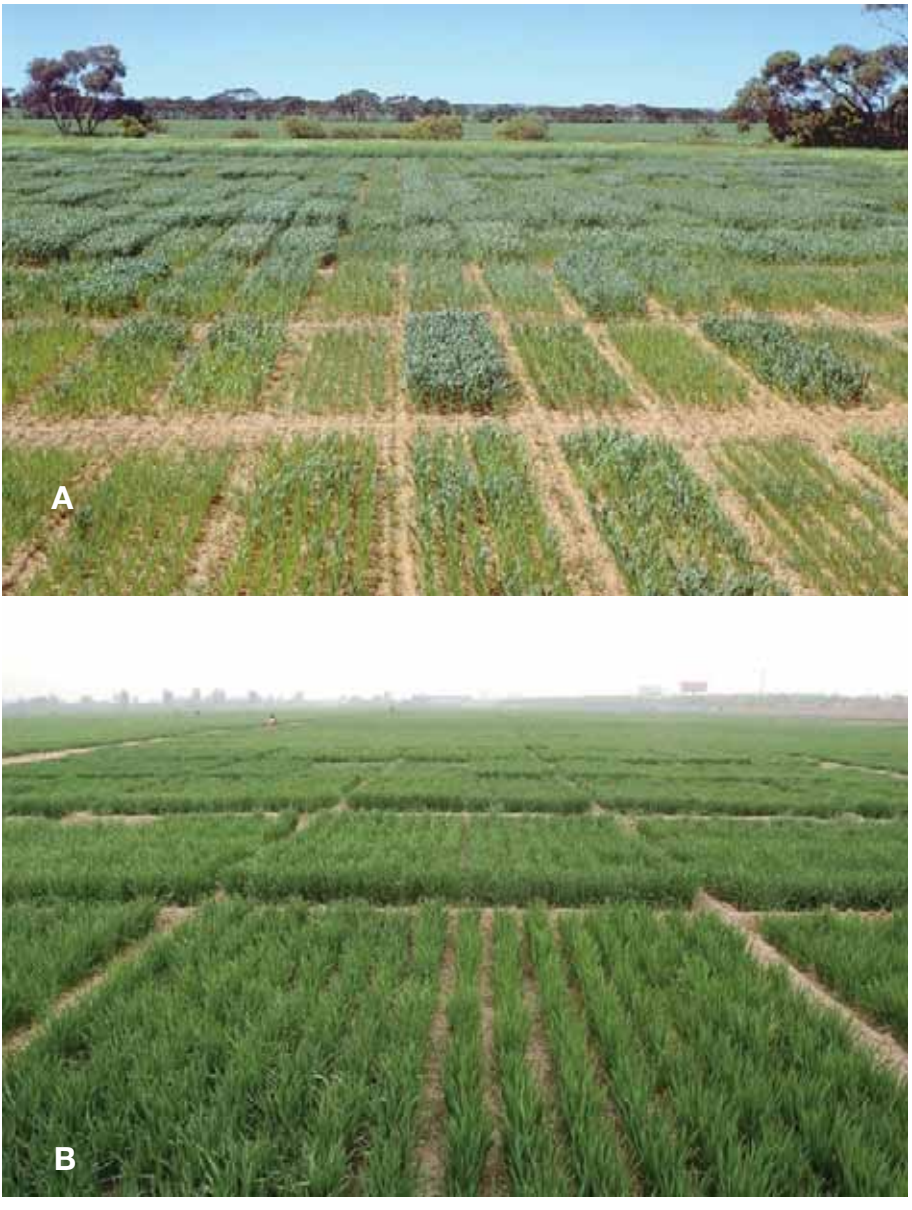

Plate 6. A, breeding program field screening for tolerance to *Heterodera avenae* at Melton, South Australia; B, field experiment to assess yield loss caused by *H. avenae* in irrigated winter wheat wheat in Hebei, China, two cultivars per bunded plots with aldicarb applied to alternate plots (Shoulong Chen). Photographs: A, Sharyn Taylor, Australia; B, Ian T. Riley, Australia.

Plate 7. A, *Heterodera avenae* resistance screening in a control environment room (tube test, see Lewis *et al.*, this volume), SARDI, Waite Campus, Adelaide; B, *H. avenae* resistance screening outdoors, single plants in tubes ('Pots' test, see Lewis *et al.*, this volume), SARDI, Waite Campus, Adelaide. Photographs: A, Sharyn Taylor, Australia; B, Ian T. Riley, Australia.

P**late 8**. A-D, extraction, collection and examination for *Heterodera avenae* cysts in samples from Qinghai, China. Photographs: A-D, Ian T. Riley, Australia.

Contents

Foreword III

Acknowledgments V

Colour plates IX

PART 1 GLOBAL STATUS

Past research on the cereal cyst nematode complex and future needs. 3
Rivoal R, Nicol JM

Longterm studies on the cereal cyst nematode *Heterodera filipjevi* in Turkey: international collaboration with regional implications. 11
Elekçioğlu İH, Nicol JM, Bolat N, Şahin E, Yorgancılar A, Braun HJ, Yorgancılar O, Yıldırım AF, Kılınç AT, Toktay H, Çalişkan M

***Heterodera avenae* and its management on wheat in India.** 17
Singh AK, Sharma AK, Shoran J

Cereal cyst nematode in Australia: biography of a biological invader. 23
Riley IT, McKay AC

Current knowledge of cereal cyst nematode (*Heterodera avenae*) on wheat in China. 29
Peng DL, Nicol JM, Li HM, Hou SY, Li HX, Chen SL, Ma P, Li Hi, Riley IT

Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in western USA. 35
Smiley RW

Global importance of the main nematodes associated with cereals in Tunisia. 41
Namouchi-Kachouri N, B'Chir MM, Hajji A

Importance and distribution of the main cereal nematodes in Morocco. 45
Mokrini F, Abbad Andalouss F, Alaoui Y, Troccoli A

Cereal cyst nematodes, root rot pathogens and root lesion nematodes affecting cereal production in Iran. 51
Tanha Maafi Z, Nicol JM, Kazemi H, Ebrahimi N, Gitty M, Ghalandar M, Mohammadi-Pour M, Khoshkhabar Zh

Present status of the cereal cyst nematode (*Heterodera avenae*) in Saudi Arabia. 56
Al-Hazmi AS, Dawabah AMM

Occurrence and distribution of cyst nematodes infecting cereals in Sicily, Italy. 61
Lombardo S, Handoo Z, Rapisarda C, Colombo A

Cyst nematodes of the genus *Heterodera* in Belgium. 66
Viaene N, Çolak Yılmaz Z, Deeren AM, de Sutter N, Vandenbossche B, Bert W

PART 2 LOCAL STATUS

Studies on morphometric variation within the population of *Heterodera zeae* on cereals in India. 73
Sharma SK, Siddiqui AU, Parihar A

Occurrence and distribution of two species of cereal cyst nematodes *Heterodera avenae* and *H. filipjevi* in Khuzestan province, Iran. 79
Ahmadi AR, Tanha Maafi Z

Occurrence of cereal cyst nematode, *Heterodera avenae* in Southeast Anatolia, Turkey. 82
İmren M, Toktay H, Öcal A, Nicol JM, Elekçioğlu İH

Status of cereal cyst nematode in wheat cropping systems and resistance in Punjab, India. 88
Kaur DJ, Sharma I, Sohu VS, Bains NS, Singh Y

Discovery of *Heterodera filipjevi* on wheat in the USA. 94
Yan GP, Smiley RW

Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. 100
Şahin E, Nicol JM, Elekçioğlu İH, Yorgancılar Ö, Yıldırım AF, Tülek A, Hekimhan H, Yorgancılar A, Kılınç AT, Bolat N, Erginbaş-Orakcı G

PART 3 MORPHOLOGY AND BIOLOGY

Molecular identification of *Heterodera* spp., an overview of fifteen years of research. 109
Waeyenberge L, Viaene N, Subbotin SA, Moens M

The life cycle of *Heterodera filipjevi* in winter wheat under microplot conditions in Iran. 115
Hajihasani A, Tanha Maafi Z

Cereal cyst nematodes in Israel, and their biology and control strategies. 118
Oka Y, Gözel U, Speigel Y, Mor M

Observations and morphometrics of cereal cyst nematode from Binzhou, Shandong, China. 124
Liu J, Wu HY, Zhang GM, Yu GL, Peng DL

Soil temperature on hatching of *Heterodera avenae* in Algeria. 130
Smaha D, Hamroun O, Mokabli A

PART 4 MANAGEMENT BY HOST RESISTANCE

Success of cereal cyst nematode resistance in Australia: history and status of resistance screening systems. 137
Lewis JG, Matic M, McKay AC

Importance of cereal cyst nematode in Rajasthan, India and its control through breeding resistance. 143
Bishnoi SP

Current global knowledge of the usability of cereal cyst nematode resistant bread wheat germplasm through international germplasm exchange and evaluation. In 'Cereal cyst nematode: status, research and outlook. 149
Nicol JM, Ogbonnaya F, Singh AK, Bishnoi SP, Kanwar RS, Li HL, Chen SL, Peng DL, Bolat N, Şahin E, Elekçioğlu İH

Management strategies for cereal cyst nematodes *Heterodera* spp. in Norway. 154
Holgado R, Andersson S, Rowe J, Clark I, Magnusson C

Identification of genetic resistance to cereal cyst nematode (*Heterodera filipjevi*) for international bread wheat improvement. 160
Nicol JM, Bolat N, Yıldırım AF, Yorgancılar A, Kılınç AT, Elekçioğlu İH, Şahin E, Erginbaş-Orakcı G, Braun HJ

Identification and utilisation of genes for cereal cyst nematode resistance (*Heterodera avenae*) resistance in wheat: the Australian experience. 166
Ogbonnaya FC, Eastwood RF, Lagudah E

Preliminary investigation of resistance of winter wheat to *Heterodera filipjevi* under controlled conditions. 172
Sağlam HD, Çobanoğlu S, Wesemael W, Nicol JM, Viaene N, AA Dababat

Use of molecular marker for screening wheat germplasm for cereal cyst nematode resistance genes (*Cre*) in Saudi Arabia. 177
Al-Dos AA, Al-Hazmi AS, Dawabah AAM, Abdel-Mawgood AA, Al-Rehiayani SM, Al-Otayk S, Motawei MI

Epidemiological studies on the Mediterranean cereal cyst nematode, *Heterodera latipons*, attacking barley in Jordan. 183
Al Abed A, Al-Momany A, Al-Banna L

Cyst nematode genomics and functional analysis of *Globodera pallida* effectors. 189
Jones JT, Chapman S, Cock PJ, Blok V

Towards developing transgenic resistance to nematodes in wheat. 191
Jones MGK, Rana J, Fosu-Nyarko J, Zhang JJ, Perera M, Chamberlain D

Chromosomal and cytological localisation of three peroxidases induced by cereal cyst nematode in wheat. 195
Simonetti E, Veronico P, Melillo MT, Bleve-Zacheo T, Delibes A, Fè Andrés M, López-Braña I

Nematode resistance in wheat transgenics expressing potato proteinase inhibitor gene (*pin2*) and its influence on plant yield and nematode fecundity. 200
Khurana P, Chhibbar AK, Sashi V, Umarao

Preliminary findings on transgenic approaches to manage cereal cyst nematode in India. 204
Umarao, Sharma A, Rao SB

Cloning of parasitism genes from the Mediterranean cereal cyst nematode, *Heterodera latipons*. 208
Lafi HA, Sadder M, Al-Banna L

PART 5 MANAGEMENT BY OTHER APPROACHES AND INTEGRATION OF STRATEGIES

Increased biodiversity in cereal cyst nematode infestations is not a threat to intensive cereal production in southern Britain. 215
Mitchinson S, Gowen SR, Kerry BR

Crop rotation and *Heterodera avenae* (cereal cyst nematode) populations in spring cereals at high altitude in Qinghai, China. 221
Riley IT, Hou SY, Chen SL

The effect of root exudates from certain Egyptian medicinal plants on cyst nematode, *Heterodera zeae*. 227
Haroon SA, Othman E, Youssef RM

Effect of solarisation on *Heterodera avenae* and wheat yield in Al-Qassim, Saudi Arabia. 233
Al-Rehiayani SM, Belal M

Impact of winter wheat, barley, broad bean and clover as preceding crops on population densities of corn cyst nematode, *Heterodera zeae* on corn in Egypt. 237
Ismail AE

Author index 242

PART 1 GLOBAL STATUS

PAST RESEARCH ON THE CEREAL CYST NEMATODE COMPLEX AND FUTURE NEEDS*

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SUMMARY

Research was initiated after World War II and intensively developed from the 1970's on cereal cyst nematode (CCN) focused mainly on the species *Heterodera avenae* in Australia, North America, western Europe and Asia. Much later, other species were reported including *Heterodera latipons* in the Mediterranean, *Heterodera hordecalis* in northern Europe and *Heterodera filipjevi* predominately in eastern Europe. Research on the distribution and economic impact demonstrate that several of these species cause important losses especially under rain-fed conditions and limited irrigation as in Australia, western Asia, north Africa, China and Pacific Northwest of the USA. Biological cycles and temperature requirements for activity of juveniles differ according to the species and populations inside species and demonstrate different hatching schemes and adaptation process to climatic conditions. Genetic relationships between populations and cereals showed a sound complexity in the host reactions with the characterisation of pathotypes of which the virulence must be known for the use of resistant cultivars. Availability of accurate techniques based on the use of biochemical and molecular markers had facilitated the identification of the species which could be found in mixtures. Combined morphologic and molecular data have improved knowledge about phylogeny of the CCN complex. Research on resistant and tolerant cultivars have identified effective sources of resistance against some species and pathotypes instead of chemical applications which had become obsolete for economical and environmental constraints. Further integrated control options have been established with various rotation schemes and the use of resistant cultivars constitute the most appropriate management options to maintain the population densities below the damaging levels. The previous global meeting on the CCN complex was held in 1982 (Rennes, France) with the participation of scientific pioneers who achieved different parts of the program presently documented. This ICCNI initiative offers the opportunity for a new set of investigators, originating

*Rivoal R, Nicol JM (2009) Past research on the cereal cyst nematode complex and future needs. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 3-10. (CIMMYT: Ankara, Turkey)

from a wider range of countries to share and tackle the challenge of CCN on an international scale.

CEREAL CYST NEMATODES

Cereal cyst nematodes (CCN) form a complex of several closely related species which are widely distributed on poaceous plants. The most reported species, *Heterodera avenae,* was described in the beginning of the 20th century (Wollenweber 1924). Description of this species was followed after the middle of this century by those of the Mediterranean *Heterodera latipons*, the north European *Heterodera hordecalis,* the eastern European *Heterodera filipjevi* and several others, to total more than 12 species (Wouts *et al*. 1995).

DISTRIBUTION AND ECONOMIC IMPORTANCE

As reviewed by Nicol and Rivoal (2008), there have been more than 15 published studies on economic damage caused by CCN on cereals. However, caution is required to compare the damage threshold from different studies due to the fact that very few studies are truly comparable, with inherent differences in environment, plant genotype, CCN species and variation in sampling protocols, extraction procedures and nematode counting. In such yield loss studies at least two key methods have been employed to assess yield loss. The first using chemical nematicides (contact and systemic) in field trials, and the second more sophisticated experiments which aimed to establish ranges of initial densities by the cultivation of crops with different hosting capacities allowed to determine the level of noxious densities (damage threshold).

As reviewed (Nicol and Rivoal 2008) and also throughout this proceedings, *H. avenae* is considered a major biotic factor affecting the wheat and barley production systems in northwestern India, several major wheat producing provinces in China and southern part of Australia. Similar results have been achieved in southern France and Spain, western Asia (Saudi Arabia), northern Africa (Algeria, Tunisia) and more recently in USA (Oregon). Economic impact of *H. latipons* was less well known but this species appeared pathogenic to barley and wheat in western Asia (Syria). The distribution of *H. filipjevi* was demonstrated wider than previously documented with losses (average 40%) on wheat in Turkey, more particularly in drought conditions. Recent field microplot studies in Iran have also indicated its economic importance (Hajihasani *et al*. 2009).

HATCHING CYCLES AND TEMPERATURE REQUIREMENTS

Published hatching studies have been reported from Canada (Fushtey and Johnson 1966), Australia (Banyer and Fisher, 1971) and also in France under both natural and *in vitro* conditions with experiments simulating seasonal variations of temperature. For *H. avenae*, two ecotypes appeared differing in the induction or suppression of dormancy (diapause) by different thermal conditions (Rivoal 1986). In Mediterranean climates the diapause was obligate, acting when hot dry conditions prevailed and being suppressed when temperature fell and soil moisture increased. Populations from more or less temperate climates had a more facultative diapause

from July to the end of winter and this was suppressed by chilling, ensuring emergence of juveniles when soil temperatures increased in the spring. Further research with north African populations (Algeria and Tunisia), demonstrated hatching schemes relevant to the Mediterranean ecotype, with higher optimum of hatching temperatures which could express adaptation of populations to warmer climatic conditions. It was demonstrated that the hatch of *H. latipons* in Syria was similar to the Mediterranean ecotype of *H. avenae* from France and southwest Spain but a population originating from a dry ecological area experienced a facultative quiescence during the summer period (Scholz and Sikora 2004). In contrast *H. filipjevi* originating from the continental Central Anatolian Plateau of Turkey does not show any diapause as the juveniles hatched immediately at the beginning of the winter wheat growing period (Şahin *et al.* 2009). Knowledge of hatching cycles for CCN has enabled a better understanding of variations in losses according to the cultivation areas. For example with *H. avenae*, synchrony between times of hatching and sowing and rainfall collectively was responsible for the great losses on winter sown cultivars in the Mediterranean regions and in contrast on the spring sown cultivars in more or less temperate climates. In addition, these results are invaluable for the production and availability of juveniles for inoculum, which enable the use of miniaturised methodology for investigations on plant resistance and genetic relationships between the CCN and their potential hosts.

GENETICS OF PLANT-NEMATODE RELATIONSHIPS

Screening for resistance in northern Europe (Sweden and Denmark) demonstrated that several cultivars or lines of oat, barley and wheat reduced or inhibited multiplication of *H. avenae* but the resistance efficiency varied according to the nematode populations. The virulence of such populations, determined by their ability to overcome resistance genes enabled the differentiation of pathotypes which were recognised using an International Test Assortment of barley, oat and wheat cultivars with respective resistance genes which was developed by Sigurd Andersen in Denmark (Andersen and Andersen 1982). Seeds of the differential hosts were further distributed in different European and Asian countries that allowed comparison of the virulence spectrum of the different nematode populations tested. The differentiation scheme devised ensured the existence of seven to eleven virulence phenotypes resulting from previous extensive selection pressure or the recent use of particular resistance genes (Cook and Rivoal 1998). When extended to populations of *H. avenae* from west Asia, studies demonstrated that additional pathotypes seemed to occur in this region showing a virulence to the three differential genes (*Rha1*, *Rha2* and *Rha3*) from barley and virulence to *Cre1* from bread wheat. Very few studies have been achieved on the virulence spectrum of *H. filipjevi* and *H. latipons* populations but preliminary results indicated heterogeneous responses more particularly to resistant germplasm in Triticeae.

IDENTIFICATION AND CHARACTERISATION

The large number of pathotypes questioned the true identity of the species involved. Species are difficult to distinguish morphologically even though improved tools are available to visualise at high magnification and take accurate measurements of specific morphological features. Controlled matings between pathotypes of *H. avenae* using the miniaturised Petri dish technique confirmed many designated

pathotypes of one species belonged to the same species. Furthermore their F2 and F3 progenies established that their genetic relationships to barley were more complex than those established previously. In the 1980s, and more recently, biochemical and molecular techniques based on the analysis of proteins and DNA polymorphism allowed a reliable identification of most species involved in the CCN complex. These analyses demonstrated particularly that several populations of the "Gotland race" (Sweden) were in fact western European isolates of *H. filipjevi*.

Until now, molecular technology failed to distinguish pathotypes of CCN and markers for virulence traits, but have established the new taxon *Heterodera australis* even though no morphological features of the cysts and juveniles differentiated this new species from *H. avenae sensu stricto* (Subbotin *et al*. 2002). Molecular markers such as RAPD and PCR-RFLP of rDNA-ITS region have enabled various CCN species to be identified, in addition to exploring the intraspecific variation in several countries as India, China or Saudi Arabia. Combined morphological and molecular data had defined phylogenetic relationships in the CCN complex and demonstrated two different and separate lineages: the *H. avenae* group containing *H. avenae*, *H. filipjevi* and the *H. latipons* group (Rivoal *et al*. 2003, Subbotin *et al*. 2003, Tahna Maafi *et al*. 2003).

RESISTANCE AND TOLERANCE IN PLANTS

Plant resistance, which is defined as a reduction/inhibition of nematode multiplication within plants, is one of the best control methods for CCN due to its wide application as it usually requires no additional equipment or cost. Ideally the resistance should be combined with tolerance (plants which have the ability to yield despite the attack of the nematode). However, the effectiveness of CCN resistance will depend on the effectiveness and durability of the resistance source and on correct identification of the nematode species and/or pathotype(s). In addition, an understanding of nematode threshold densities that result in yield loss and the interaction of these thresholds with biotic and abiotic factors is required.

As mentioned above one of the most important factors in understanding resistance is to classify the species and pathotype(s) of populations of interest. To date, almost all studies have been achieved with the more commonly reported *H. avenae* using the International Test Assortment of barley, oat and wheat was developed by Andersen and Andersen (1982). However, as mentioned this test is more than thirty years old and does not cater for the wider variation of species and pathotypes which are presently reported. Very few studies have been conducted on the two other species *H. filipjevi* and *H. latipons* but preliminary research indicated heterogeneous responses between populations to different resistant germplasm. It was also demonstrated that populations of *H. avenae* differed in the capacity of juveniles to produce female (part of the fitness component) which was important for designing virulence/resistance investigations and for the management of nematode densities (Rivoal *et al*. 2001).

A summary of the currently published CCN sources and their genetic location is reviewed in Nicol and Rivoal (2008). The identified sources of resistance to *H. avenae* have been found predominantly in wild relatives of wheat in the genus *Aegilops*, with six out of the seven named *Cre* genes for *H. avenae* resistance coming from four *Aegilops* spp. and these have already been introgressed into

hexaploid wheat backgrounds for breeding purposes. The breeding of resistance has been relatively efficient as in almost all cases the genetic control of resistance with CCN has been defined by a single dominant gene. However, the effectiveness of these designated *Cre* genes is dependent on both the species of CCN and pathotype. Work is ongoing in many countries to determine the effectiveness of the known *Cre* genes against several species and pathotypes of CCN (Nicol *et al.* 2009a,c) and also identify and characterise new sources of resistance. Most recently CIMMYT and Turkey in collaboration have established a new host differential with the currently known hexaploid wheat sources of resistance which has been distributed to more than 10 countries. It is essential, but clearly a challenge to understand the usability of resistance in different regions and requires a coordinated network of participating scientists and countries.

Once effective sources of resistance are identified the use of markers for both wheat and barley in a marker-assisted selection program is possible to pyramid resistance genes against CCN. This is actively being achieved in Australia and within the ICWIP (ICARDA-CIMMYT Wheat Improvement Program) for some of the known wheat and barley genes which have been further characterised. In the future, it may be possible to transform wheat using resistance genes as a method to produce nematode resistant wheat cultivars.

MANAGEMENT OF POPULATIONS AND INTEGRATED CONTROL

In many countries where these nematodes occur wheat is often one of the major food staples, and the control of the nematode is of considerable importance to improve the production and livelihood of the farming communities. Furthermore much of west Asia and north Africa is characterised by wheat monoculture systems, where rainfall or irrigation is limited and options for crops rotation are not used or restricted. Such cropping systems frequently suffer moisture or drought stress and in these environments the effects of the nematode damage can be increased, and hence control of nematodes in these cropping systems is of paramount importance.

Many different control options such as chemical, cultural, genetic (resistance/tolerance) and biological control are available and their net effect should be aimed at decreasing and maintaining CCN population densities below damage thresholds, so as to maintain or reach the attainable yield. However in order to achieve this a clear understanding of nematode threshold densities that result in yield loss and the interaction of these thresholds with biotic and abiotic factors is required.

Cultural practices represent efficient methods based on rotational combinations of non-hosts crops or cultivars and clean fallows. Frequencies of such combinations should be calculated upon data inferring from specific studies of population dynamics according to the targeted inputs. Application of fertilisers and soil amendments may compensate the reducing effect of nematodes on wheat yields but their use is frequently limited by financial constraints. Adjustment of sowing dates to escape synchrony of peak emergence with the more sensitive stage of the crop could maximise the final yield. Trap cropping could constitute efficient measures to decrease nematode densities. Allelopathy techniques based on toxic plant root exudates and microbial secretions offer also some alternative controlling measures.

In the past, low rates of nematicides applied to both soil and seed provided effective and economical control (e.g. in Australia, India and Israel), however the present day cost and environmental concerns associated with these chemicals do not make them a viable economic alternative for almost all farmers. Their use in scientific experiments to understand the importance of these nematodes will nevertheless remain vital.

The use of resistant/tolerant cultivars which ensure both reduction/inhibition of nematode multiplication offers one of the most effective control methods with no additional cost or equipment in addition to be environmentally friendly. However, the use of resistant cultivars requires a sound knowledge of the virulence spectrum of the targeted species and pathotypes. Engineering of transformed plants with inhibitors to the development of nematodes may be part of the future options for some countries.

The prospects for using biological antagonists within an IPM strategy for wheat nematodes is still considered promising with the development of natural populations of enemies (e.g. *Verticillum* and *Nematophtora*) or application of exogenic pathogens *(Trichoderma viride*). However, their ultimate use relies greatly on the agroecology of the cropping systems for persistence and effectiveness which may be appropriate in more optimal cropping systems.

TRAINING AND INTERNATIONAL NETWORKS

One of the important considerations is to ensure capacity development of scientists working on CCN. In many countries the more common diseases of wheat such as rust are well understood and many trained scientists are actively working. Significant efforts over the last six years with CIMMYT, ICARDA and national programs in Turkey, China and Tunisia, have enabled international tailor made training which has been supported by many donors especially The ATSE Crawford Fund of Australia. This has enabled many groups to activate and enhance their research efforts on CCN (Nicol *et al*. 2009b). In 2006 under the leadership of CIMMYT the International Cereal Cyst Nematode Initiative (ICCNI) was formed with the first meeting hosted by INRA headquarters in Paris France. The groups represented at this meeting include Belgium, France, United Kingdom, Turkey and China. The mission of the ICCNI was defined as to join efforts to create a critical mass of scientific capacity and skills to deliver sustainable solutions by working at both the applied and theoretical level. The key objectives of the ICCNI with partners were defined: 1, understand the importance and distribution of CCN; 2, investigate potential control options with a major emphasis on host resistance - both gene discovery, validation and integration of resistant sources (traditional and molecular) into wheat germplasm for global wheat production systems; 3, train and capacity build national program scientists from developing countries.

FUTURE PROSPECTS

CCN is acknowledged as a global economic problem on cereal production systems. Global warming will enhance dramatically the noxiousness of these pathogens in both dry land and rain-fed production of cereals as well as in intensive production systems in western Europe. Although substantial progress has been made, it is clear

that additional studies are needed to evaluate the economic importance of CCN in developing countries (North Africa, eastern and western Asia) and also in developed countries (western and eastern Europe, USA) which face both greater climatic constraints and reduced fertiliser utilisation. A main research challenge will concern the genetic diversity of these species and populations, their phylogenetic and phylogeographic relationships, as recently achieved on another cyst nematode (Picard *et al*. 2007). Research must confirm sustainable management solutions which require deeper understanding of the population dynamics of *H. avenae* and other species. Active research on resistance sources associated with their molecular characterisation is necessary for more rapid integration into cereal cultivars using existing and new tools as they become available. A new and important challenge is offered to young and older nematologists and associated disciplines involved in traditional or more advanced scientific skills, originating from developed and developing countries. Given the forecasted future need for cereals it is essential that interdisciplinary and international networks such as the ICCNI strengthen to enable the formation and strengthening of networks to find practical solutions to a significant biotic constraint affecting food security in particularly the rained wheat productive systems.

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LONGTERM STUDIES ON THE CEREAL CYST NEMATODE *HETERODERA FILIPJEVI* IN TURKEY: INTERNATIONAL COLLABORATION WITH REGIONAL IMPLICATIONS*

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SUMMARY

Globally cereal cyst nematodes (CCN) are economically important in wheat production systems. Preliminary work started in 1993 establishing the wide spread distribution of cereal nematodes in both East Mediterranean region and Central Anatolia of Turkey. In 2001, a new joint initiative was established between CIMMYT International, the Turkish Ministry of Agriculture and Çukurova University to understand: 1, the identification and distribution of *Heterodera* species in main rain-fed wheat production areas; 2, the economic importance and improve our understanding of the some biological parameters such as hatching of CCN; 3, screen and assess known sources of resistance and identify new sources; 4, integrate new sources of resistance into bread wheat cultivars for Turkey and International germplasm; 5, investigate other integrated pest management options such as rotation and different wheat management strategies. Key results indicate CCN is widely distributed in the rain-fed wheat production systems and causes significant yield loss on common cultivars. Effective control can be achieved with the use of non-host rotation (legumes, safflower), although this option is limited in the winter wheat production areas of Turkey. Genetic host resistance has been identified in a number

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of Turkish and International wheat germplasm and offers one of the most environmentally, cost effective and sustainable control options. The ongoing project of CIMMYT and Turkey will continue developed and disseminate nematode disease resistant germplasm for the region (west Asia, north Africa and other global regions of importance).

INTRODUCTION

The *Heterodera avenae* group consists of 12 valid nematode species which are parasitic on cereals. The most economically important species reported on cereals are *H. avenae*, *H. latipons* and *H. filipjevi* (Abidou 2005, Nicol and Rivoal 2008). Rumpenhorst *et al*. (1996) identified the cereal cyst nematodes (CCN) as *H. filipjevi* in Central Anatolian Plateau (CAP) in Turkey. Primarily results indicated that *H. filipjevi* was found in 78% of the wheat growing area in the CAP and transition zone in Turkey (Şahin *et al*. 2009b).

SURVEYS

An intensive survey of 286 soil samples was collected from 23 provinces of CAP during the 3 seasons in March 2003-2005. CCN were identified in basis of the vulval sections and second stage juvenile morphometrics and morphological characters and also using molecular PCR-RFLP technique.

Heterodera cysts were found in 78% of soil samples and in all locations except one CCN were identified as *H. filipjevi*, whilst one population in Yozgat was identified as *H. latipons* (Şahin *et al.* 2009a). *H. filipjevi*, is increasingly being reported around the world after being distinguished from *H. avenae* in Tadjikistan by Madzhidow (1981) from USA (Smiley *et al*. 2008), Norway (Holgado *et al*. 2004), Germany (Große and Kohlmüller 2004), Iran (Tanha Maafi *et al*. 2003), Sweden (Cook and Noel 2002), India (Bishnoi and Bajaj 2002), Turkey (Rumpenhorst *et al*. 1996), Bulgaria, England, Poland, Estonia, Spain (Subbotin, *et al*. 2003) and Russia (Balakhnina 1989). In the past *H. avenae* has been considered the most important CCN species, however it would appear in the last decade *H. filipjevi* is widespread and more suited to continental environments.

HETERODERA FILIPJEVI HATCHING AND POPULATION DYNAMICS

In vitro hatching of *H. filipjevi* was investigated under laboratory and field conditions. Five temperatures of 5, 10, 15, 20 and 25C were used in laboratory experiments. Similarly, the development of CCN *H. filipjevi* was investigated under rain-fed cereal conditions over three growing seasons (2002-2005) on the winter wheat cultivar Bezostaya.

Under in-vitro conditions, highest hatching percentages were obtained with 15, 10 and 5C treatments; 94, 92 and 75%, respectively. Hatching at 20 and 25C treatments was low; being 22 and 19%. Hatching significantly increased with the temperature change from 5C to 20C and from 10C to 20C at the rate of 49 and 42%, respectively (Şahin *et al*. 2009a). Hatching of same population (TK1 *H. filipjevi* from Haymana) followed under natural field temperature conditions during the field season found

optimum juvenile hatching is below 20C and hatching increases with the change from lower temperatures to high temperatures. Juvenile emergence of *H. filipjevi* was recorded during the winter period just after sowing from November to March being correlated with the lowest temperatures. Mature white females were found on roots at the beginning of May during wheat stem elongation and head development and mature cysts appeared later on. The total number of cysts and eggs in the soil had only one peak at the end of each growing season, suggesting that *H. filipjevi* was monocyclic. Multiplication rates were inversely correlated with initial nematode densities with ceiling levels of between 15 and 20 eggs/g of dry soil (Şahin *et al*. 2008).

YIELD LOSS

Unlike the more studied *H. avenae*, little is known about the economic importance of the species of *H. filipjevi*. Yield loss trials have been conducted over two consecutive years (2002/03 and 2003/04 seasons) in two locations (Haymana and Cifteler) on the CAP under natural field conditions (Nicol *et al*. 2006 and unpublished data).

The average yield loss (comparing treated and untreated nematicide plots) in 2002/03 was 20% for Cifteler and 36% in Haymana across the twelve varieties. The two commonly cultivated winter cereals Bezostaya and Gerek suffered high losses of 47 and 37%, respectively. Clear significant negative regressions were found with *H. filipjevi* initial population and yield loss with cereal varieties providing some basis to understanding the economic threshold for loss, however these were found to differ greatly under varied environmental conditions. Considering the drier year initial (2002/03) densities between 5-10 *H. filipjevi* eggs/g can be considered economically damaged (causing >10% yield loss). This work is fundamental, but requires mutli-location and year testing factoring in the key environmental factors and their relation to yield loss.

ROTATION

A long-term rotation trial established on the Haymana (Ankara) İkizce experimental station since 1975 has been monitored in March 2003-05 for populations of cyst nematode under the different rotational combinations. A range of rotational regimes were applied including chickpea, fallow, safflower, spring lentil, sunflower, wheat, vetch/barley mix, winter lentil and winter vetch.

The fallow/wheat rotation for cyst nematode was found to show no difference when compared to wheat/wheat rotation, inferring fallowing does not significantly reduce cyst populations. In order of hosting ability for cyst nematode over the three years vetch/barley>wheat>fallow>chickpea, sunflower, spring lentil, winter lentil, winter vetch>safflower. From this study fallowing is considered not effective method to reduce cyst populations. Barley should be avoided under cyst populations are high (Elekçioğlu *et al*. 2004).

CCN RESISTANCE IN CIMMYT-TURKISH GERMPLASM

The most effective method to screen for resistance is under controlled greenhouse conditions with mass culture of CCN. Adopting such published methods this work has been successfully initiated in Turkey over the past five years (Nicol *et al*. 2007). More than 1,000 national and international winter and spring wheat lines and cultivars from Turkish National and International (CIMMYT and TCI (TURKEY/CIMMYT/ICARDA) and other International) were screened in order to determine their resistance against *H. filipjevi* local isolate TK1 from Haymana. Furthermore the known published *Cre* genes available in bread wheat background for *H. avenae* resistance were also screened. Two moderately resistant (Sönmez, Silverstar or Katea) and susceptible lines (Bezostaya 1 and Kutluk) were used in each test to compare data from ANOVA.

Repeated screening has identified 28 lines to have resistance as good as or better than the known control lines (Nicol *et al.* 2009). Of the known published *Cre* genes, *CreR*, *Cre1* and the Milan VPM source - *Cre5* (also possibly *Cre2* and *Cre6* genes) was found to provide some level of moderate resistance, while others tested (*Cre3* and *Cre8*) were found to be ineffective. Many of the lines identified are in high yielding adapted spring and winter wheats, and several represent Turkish released cultivars. Further work is needed to validate this resistance under natural field conditions. As Nicol and Rivoal (2008) indicate the effectiveness of designated *Cre* genes depends on both the species of CCN and pathotype. As CCN is a known regional problem in west Asia and north Africa a quarantined laboratory has been established in Turkey at Çukurova University which will enable testing of the most promising resistance material identified in Turkey against *H. filipjevi* to determine if they are effective against isolates of CCN from partner countries.

MARKER-ASSISTED SELECTION FOR CCN RESISTANCE

A published known single dominant gene designated *Cre1* for resistance to a closely related species *H. avenae* (Ogbonnaya *et al*. 2001) was assessed in Turkey for its resistance in wheat, and was found to be effective. As a result the IWWIP (International Winter Wheat Improvement Program, Turkey, CIMMYT and ICARDA) breeding program has incorporated this source of resistance from the Australian spring bread wheat cultivar Silverstar into highly adapted winter wheat backgrounds. The *Cre1* marker was optimised using the technique and optimised using STS-PCR method (Akar *et al*. 2009). This method was applied in a number of segregating winter wheat populations from F2-F4 containing Silverstar. Preliminary results indicate marker-assisted selection is functioning effectively with a recovery of *Cre1* positive lines up to 88% depending on the cross. These marker positive lines are now being integrated into the breeding program. This tool has the potential to be used effectively on a larger scale to incorporate CCN resistance into wheat as has been achieved routinely in other breeding programs such as Australia. Furthermore, a number of Turkish germplasm have been checked for the presence of *Cre1* gene, however to date none of germplasm have tested positive (Akar *et al*. 2009).

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HETERODERA AVENAE AND ITS MANAGEMENT ON WHEAT IN INDIA*

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SUMMARY

Cereal cyst nematode (CCN), *Heterodera avenae*, was identified in India in Sikar, Rajasthan by Prasad and coworkers in 1958 and this put nematology at the national level. This nematode is globally distributed and causes significant economic losses under stress conditions. Its occurrence in Roorki (Haridwar, Uttarakhand), Jhansi (Uttar Pradesh) and Sagar (Madhya Pradesh) suggests prospective likelihood of establishing in Indo-gangetic plains. To date, CCN has caused heavy losses to farmers in the endemic areas like Rajasthan, Haryana and others. Yield loss up to 47% due to CCN in sandy soils of Rajasthan during late 1960s and early 1970s has been reported for wheat cv. Kalyansona. Five biotypes of *H. avenae* have been reported from Rajasthan and Haryana but only two biotypes have been authenticated. Subsequently, the existence of two species namely, *H. avenae* in Rajasthan, southern Haryana and MP whereas *H. filipjevi* were reported in northern Haryana, Punjab and Himachal Pradesh. Besides these, southern and northern populations of CCN in Haryana continue to found as mixed population of *H. avenae* and *H. filipjevi*. Five juveniles/g of soil is sufficient to cause economic damage. Infested patches are frequently seen in free draining loamy soils and the population increases when these infested patches are cropped with wheat and other host cereals. Infestation of CCN in poorly drained soils are less common. The impact of cropping systems like rice-wheat, cotton-wheat, maize-wheat, groundnut-wheat, pearl millet-wheat on CCN management has been expensively studied.

INTRODUCTION

The green revolution enabled food production to increase from 50.8 Mt in the year 1950 to 230 Mt in the year 2007 ensuring stable per capita availability of food grains. Our population is still growing at the rate of about 2%. The shortfall in food grain production in India is expected to be between 15 and 20 Mt by the year 2020. To realise the projected production of about 250 Mt by 2020, the average food grain

*Singh AK, Sharma AK, Shoran J (2009) *Heterodera avenae* and its management on wheat in India. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 17-22. (CIMMYT: Ankara, Turkey)

productivity has to increase from the present 1.7 to 2.0 t/ha by 2020. Overall in India, agriculture is a way of life accounting for nearly 70% of the country's employment, about 17% of the GDP and nearly 20% of export earnings. Nearly 600 million farmers in rural India are involved in agriculture activities. With about 2.3% of the global land area and about 4% of world's water resources, India supports 17% of the global population and 15% of world's livestock. A well developed agricultural research system, a significant area under irrigation (around 60 Mha) and increased productivity in major crops enabled Indian agriculture to become globally competitive. Presently, India is one of the largest producers of rice, tea, milk, coffee, cotton, fruits, and animal products in the world. In view of stagnating food grain production and increasing consumption need of the growing population, the Government of India has launched a centrally sponsored scheme National Food Security Mission (NFSM) to increase production and productivity of wheat, rice and pulses on a sustainable basis so as to ensure food security of the country. The approach is to bridge the yield gap in respect of these crops through dissemination of improved technology and farm management practices. The implementation of the NFSM would result in increasing production of rice by 10 Mt, wheat by 8 Mt and pulse by 2 Mt by 2011-12. The mission covers 133 districts of 12 states under NFSM-Rice, 138 districts of 9 states under NFSM-Wheat and 168 districts of 14 states under NFSM-Pulses. It would also create additional employment opportunities. It implies more pressure on our existing land, soil and water resources which are already in short supply, degraded, fragile and this similar situation is being faced by mainly developing countries today. Although many nematodes have been found associated with small grain cereals, but only a few of them are considered economically important. This includes CCN (*Heterodera avenae*) in wheat and barley. Recently, researches have determined fact that a incidence of nematode populations occurs in the rhizosphere of wheat crops, with *Heterodera* spp. being the most prevalent.

LOSSES

As per trials reported in 1960s, CCN caused losses worth Rs 40 million and Rs 30 million in wheat and barley, respectively in Rajasthan. Little is known about the economic importance of the species *H. filipjevi,* but it is assumed that it can cause damage up to 20%. CCN, *H. avenae* is a root pathogen of cereal especially wheat and barley that can cause sever losses in intolerant wheat cultivars. The sedentary soil borne CCN is an important biotic constraint limiting the productivity and production of wheat and barley crops in sandy soil of semi-arid areas of Rajasthan and Haryana. To date, this nematode has been causing heavy losses for farmers in the endemic areas in India (particularly Rajasthan and Haryana). Losses up to 47% have been reported in wheat cv. Kalyansona in sandy soils of Rajasthan during late sixties and early seventies. Broad leaved crops like fenugreek, onion, carrot, mustard and gram helped in reducing the population of CCN by 47-55% in first year and up to 75% by the end of second year. However, these crops are not popular with farmers due to marketing problems and established dietary preferences, though wheat yield increased by 83% after one year and 134% after two years of rotation in the infested fields, which were yielding not more than 1 t/ha previously. A combination of 3 to 5 cultivations along with a chemical pesticide (carbofuran at 1.5 kg a.i./ha) has been shown to effectively control this pest. Lesser doses of carbosulfan, phenamiphos, in combination with bioagents and neem oil were tried

but still carbofuran continued to be the most effective. Development of CCN resistant cultivars remains the prime objective for CCN control in the endemic areas. CCN resistant barley cvs Raj Kiran, RD 2508, RD 2035 and RD 2052 were developed, and cvs BH 331 and BH 338 were identified to have a substantial degree of resistance against *H. avenae* but some of them exhibited little resistance against *H. filipjevi* and other *Heterodera* spp.

BREEDING MATERIALS AND THEIR UTILISATION IN DEVELOPMENT OF CCN RESISTANT CULTIVARS

Breeding for resistance and/or tolerance to *Heterodera* spp. is one of the most economical and effective ways to help farmers manage the problem, but it is not the only way. The scientists are using every tool at their disposal to identify resistant wheat and barley lines and provide them to breeding programs. Wild relatives of wheat have resistance to some nematodes. The resistance of these is being studied throughout the world. It considered that CCN losses will decline significantly if effective rotations and the widespread adoption of well adapted cereal cultivars with suitable resistance are deployed. However, identification of consistent sources of resistance in wheat has not been possible despite the screening of thousands of genotypes in India alone (Dhawan and Nagesh 1987). There is need to search sources of resistance to this nematode among wild relatives of wheat. One of the most cost effective and sustainable methods of control is the use of host resistance. To date, more than 9 single dominant genes known as *Cre* gene family has been found worldwide with many of these originating from wild relatives of wheat. In order to bring excellence in breeding program, a sufficient understanding of the number of species and pathotypes within species is essential. Although useful, a pathotype scheme for a species complex based on interaction with these cereal genera will not easily describe extensive variation in virulence. Furthermore, there are few molecular or other diagnostic methods that can provide consistent and reliable pathotype differentiation. For now, the established approach is still employed being convenient and adequately reliable.

ALTERNATIVE APPROACHES IN MANAGING CCN

Crop rotation

To reduce the impact of *H. avenae*, growing non-host crops after host crops to reduce nematode population density may prevent significant damage to cereal crops. Attempts were made to manage populations by fallow and growing non-host crops because CCN is host specific to cereals. One study indicated that nematode population densities decreased by 70% with continued rotation with non-host crops like mustard, carrot, fenugreek, onion and gram, or by fallow, summer ploughing and other means. With two year's rotation with non-host crops, the yield increased by 87%.

Manure

Organic manure influence the development of *Heterodera* spp. as farmyard manure (FYM), compost and saw dust application resulted in improved growth and discouraged the multiplication of CCN.

Irrigation

Higher multiplication rates of CCN in well irrigated wheat and barley, compared situations with with poor soil moisture, have been observed.

Summer ploughing

Soil temperature during May-June generally remains more than 30C and goes up to 48C. By ploughing, the soil is loosened and freshly formed cysts are disturbed and with the turning of the loose soil they are brought closer to the surface. As the moisture in the soil evaporates and cysts are subjected to dry heat. The nematode eggs with the cysts are sensitive to such desiccation, and many will perish.

Agrochemicals

Earlier soil fumigants like DD (1,3-dichloropropene/1,2-dichloropropane) at 300 L/ha and DBCP (dibromochloropropane) at 45 L/ha were used. Later granular nematicides like aldicarb 10G and carbofuran 3G (both at 1.5 kg a.i./ha) were used which gave good protection against CCN and also gave better plant growth and increased grain yield (Handa *et al.* 1980, Mathur and Handa 1984). Alternative to exclusive chemical management strategy of CCN, concerted efforts were made by evaluating different organic, inorganic, resistance sources and biological agents in combination during the wheat crop seasons of 1997-2005 (8 years) in order to keep CCN populations below damage threshold level in endemic and hot spot areas.

Though other treatments, like wheat cv. CCNRV-1, carbofuran in isolation reduced the number of females per plant but combination involving *Trichoderma* spp., FYM, and reduced rate of carbofuran proved better at Durgapura. At Karnal, *Trichoderma viride* in combination with VAM fungi gave substantial growth and reduced the number of females/plant to 5.4 compared to 20.9 in the untreated control. When this exercise was repeated at Durgapura, *T. viride* plus FYM and carbofuran 3G (1 kg a.i./ha) had yielded similarly to carbofuran (1.5 kg a.i./ha) giving 4.1 t/h and 4.2 t/h and reduced the number of females to 2.9 and 3.0 per plant, respectively. By comparison the untreated control yielded 2.4 t/ha and had 18.5 females/plant. However, the treatment involving *T. viride* plus FYM also gave significantly higher yield of 3.6 t/h and reduced number females to 6.8/plant, compared to a yield of 2.4 t/ha and 18.5 females/plant in the control.

At Hisar, all the treatments inhibited nematode multiplication compared to control. Grain yield and shoot biomass increased for FYM (20 t/ha), Posse ST (0.75 kg a.i./ha) plus FYM (20 t/ha), seed treatment with *T. viride* plus FYM (10 t/ha), seed treatment with *T. viride* plus FYM (10 t/ha) and carbofuran 3G (0.75 a.i./ha), and seed treatment with *Gliocladium virens*. Carbofuran 3G (1.5 kg a.i./ha) and seed treatment with *Trichoderma harzianum* increased yield but not biomass.

At Karnal, *T. viride* (4 g/kg) seed plus carbofuran 3G (1 kg a.i./ha) was the best after carbofuran (1.5 kg a.i./ha). In the subsequent year at Durgapura, three treatments, carbofuran 3G (1.5 kg a.i./ha), compost plus *T. viride* and half dose of carbofuran 3G (0.75 kg a.i./ha), gave the highest yield of 4.4 t/ha, and also reduced the population significantly to 2.3 and 2.7 females/plant, respectively, compared to untreated control (yield 2.1 t/ha and 5.1 females/plant). In addition, treatment with compost and half dose carbofuran, neem seed powder or the resistant cv. CCNRV-1 also gave significantly higher yield, 4.1, 3.8 and 4.1 t/ha and also reduced the CCN

to 2.7, 2.9 and 0.7 females/plant, respectively. By comparison, the untreated control yielded 2.1 t/ha and had 5.1 females/plant. It was observed that *T. viride*, when used alone as seed treatment, did not produce significantly higher yield but gave slight reduction in CCN numbers. At Hisar, all the treatments, except seed treatment with *Azotobacter chroococcum* at 4%, proved effective in reducing the CCN number compared to the control. Seed treatment with either *A. chroococcum* or *T. viride* along with soil application of carbofuran 3G at 0.75 kg a.i./ha was as effective as carbofuran 3G at 1.5 kg a.i./ha. These findings suggest that *T. viride* in combination with compost and half dose of carbofuran can reduce substantially the CCN population and improve the health of soil (Tandon and Sethi 1986, Directorate of Wheat Research 1999-2005).

Biotypes

Five biotypes of *H. avenae* have been reported from Rajasthan and Haryana but recently two biotypes were authenticated. Bishnoi and Bajaj (2004) reported the existence of two species in India namely, *H. avenae* present in Rajasthan, southern Haryana and MP, and *H. filipjevi* in northern Haryana (Ambala, Morni Hills), Punjab and Himachal Pradesh. In southern and northern Haryana mixed population of *H. avenae* and *H. filipjevi* occur.

Populations

Five juveniles/g of soil is sufficient to cause yield loss and with increasing number of juveniles, the severity of the damage increases, as damage is density dependent. Infested patches are seen frequently in free draining loamy soil and population gets larger when these infested patches are cropped with cereals. Infestation of CCN in poorly drained soils are less common. Cropping systems like rice-wheat, cotton-wheat, maize-wheat, groundnut-wheat, pearl millet-wheat have been studied and their impact on CCN determined.

Novel approaches

This pest can be managed effectively through a concerted and integrated approach. There are examples around the world where CCN population had declined significantly in the last two decades and ultimately wiped out due to more effective rotations and resistant wheat cultivation. Host resistance seems to be the most effective approach in managing this menace. However, several thousand advanced genotypes and breeding lines were screened against CCN under the All India Wheat & Barley Improvement Project, but none showed resistance across all locations due to pathotype variation, species and/or biotype variability and limited resistance genes in the material. For this reason higher priority is given to disease problems like rusts, Karnal bunt and foliar blights. CCN resistant wheat cv. Raj MR 1 with a single dominant gene, developed by crossing of AUS 15854 (a Turkish wheat) with the local cultivar J 24 was found to be highly susceptible to yellow rust, which is the major disease problem in the region. Hence, there is a need to introduce CCN resistance to wheat along with resistance to the major disease problems, like yellow and brown rusts. In fact, in India, no cultivar is recommended for release and cultivation unless it possesses a high level of resistance against rusts. Thus efforts need to be directed in locating new sources of resistance gene against CCN in bread wheat, related wild species from west and central Asia and to introgress them in Indian cultivars.

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CEREAL CYST NEMATODE IN AUSTRALIA: BIOGRAPHY OF A BIOLOGICAL INVADER*

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SUMMARY

Heterodera avenae (cereal cyst nematode, CCN) is an invasive pest of old-world cereals, which probably entered and established in Australia in the 1800s. It spread widely in southeastern Australia, with outliers in other areas, but did not reach its full impact till the 1960s. Early control was through agronomic means but as impact bordered on disaster, the focus shifted to chemical control and then to host resistance. Resistance is now widely deployed, with over 50% of current cultivars moderately to strongly resistant. *H. avenae* population densities and detections have declined markedly over the last decade. This success rests on Australia having a single pathotype. To maintain this success, resistance must continue to be deployed, and quarantine and surveillance are needed to minimise the risk that it is compromised by the invasion or development of other CCN species or biotypes.

INTRODUCTION

Australia is an island continent with a diverse and unique flora that evolved in relative isolation from the rest of the world. Over some 60,000 years occupying the land, the original inhabitants did not develop animal husbandry or agriculture. For several centuries before European colonisation, seafarers from Sulawesi visited the coast of northern Australia to fish for sea cucumber and trade with aboriginal communities to supply the markets of southern China. However, this did not result in the introduction of cultivated plants or the transfer of agricultural practice.

The introduction of crop plants began with European colonisation of Australia, and in parallel, the unintentional introduction of crop pathogens, pests and weeds. In 1788, where Sydney now stands, the first English settlement was established. The first grain crop was harvested at Rose Hill (now Parramatta) in 1789 (Connah 1993). England was midway though 100 years of rapid expansion in population and in the

*Riley IT, McKay AC (2009) Cereal cyst nematode in Australia: biography of a biological invader. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 23-28. (CIMMYT: Ankara, Turkey)

production of wheat, barley, rye and oats, to supply the growing demand in a protected domestic market (Henzel 2007). So in England's southernmost colony, wheat became the focus of early cropping pursuits. Maize, although easier to grow in the roughly-cleared, poorly-tilled fields of the new colony, was largely unknown in England and regarded, even by convicts, as food fit only for the impoverished (Karskens 2009). South Australia (SA) soon became the major wheat producing State, with free settlers many of whom had a farming background, moderately fertile red-brown earths and more easily cleared native vegetation (Henzel 2007). Over the next two centuries, wheat (Figure 1) and cereal production became a major land use in five mainland States, with forecast production of wheat, barley, oats and triticale in 2009/10 at 32 Mt from 19 mil ha (Anon. 2009).

Figure 1. Australian wheat growing areas (Anon. 2009, used under provisions of the Australian Copyright Act 1968).

It is likely that wheat and other cereals were brought to Australia with a complement of pests and pathogens, but others have arrived since. This biological invasion continues, despite the establishment of robust quarantine measures (Eagling 2009). Most, if not all, pathogens causing economic damage to cultivated plants are introduced, having commonly co-evolved with their hosts.

Cereals cyst nematode (CCN, *Heterodera avenae*), a damaging parasite of wheat, barley and oats exemplifies this process. Its host crops originate from west Asia or northern Europe and so too would most of their nematode parasites. In the context of growing interest in invasion biology and biosecurity, which now are established as new and independent disciplines of biology (Davis 2009), and the importance of wheat in Australia (Figure 1), we have attempted to provide a brief history and analysis of CCN as a biological invader in Australia. This story spans CCN's rise as a major constraint to crop production to its exemplary control by host resistance, and the need for continued vigilance in its management and the exclusion of other pathotypes and related species.

HISTORY

It is almost certain that the incursion, establishment and initial spread of *H. avenae* went unnoticed, and even if it was detected early, it is unlikely that the significance and eventual impact could have been imagined at that time. The relatively recent incursions of *Globodera rostochiensis* in Australia (Quader *et al.* 2008) highlight the problem with early detection of root nematodes, even in a modern, intensive industry.

McLeod (1992) reviewed the possible introduction and spread of *H. avenae* in Australia, and considered the first evidence to be a herbarium specimen from SA dating to 1904 (Meagher 1972). The first recognition of CCN as a pest of cereals in Australia was in 1930, also in SA (Davidson 1930). CCN became established through much of the cropping area of SA and western Victoria. By the 1960s CCN had become a major economic concern (Robinson 1961) and by the 1980s, seriously undermining the economic viability of many farming enterprises, with 2 Mha known to infested and losses estimated at A$72 mil./year (Brown 1984). Eventually, CCN was found in New South Wales (McLeod 1968), more widely in Victoria and in Western Australia (Parkin and Goss 1986); this process considered to represent long-distance spread within Australia rather than new introductions (McLeod 1992).

Australian populations of CCN were identified as *Heterodera avenae* (Meagher 1974) and considered to be most closely related to those from northern Europe (Meagher 1977, McLeod 1992), on the basis of morphology and ecology.

Following the first recognition of CCN as a damaging pest in Australia (Davidson, 1930), early efforts to develop control (e.g. Garret 1934, Fisher 1966, Meagher 1968) focused on cultivation, crop rotations and nutrition. An overview of this history was recently provided by Vanstone *et al.* (2008). In the 1960s the focus shifted to nematicides and predictive bioassays (Brown 1987). However, as the impact of CCN remained uncurbed, the focus again shifted in the 1970s to early 1980s, this time to resistance. Rathjen *et al.* (1998) relate the events that led to greater recognition by breeders of the need for and benefits of resistant cereals adapted for southeastern Australia. The widespread use wheat cv. Festiguay in response to rust epidemics was a fortuitous contributor to this as it happened to be CCN resistant.

Subsequently the resistant, well-adapted barley cv. Galleon (Sparrow 1981) was released and the course to widespread and effective control of CCN in Australia was set. Since the release of Galleon, an increasing number of resistant cultivars of wheat, barley and oat have been bred, released and widely adopted (Rathjen *et al.* 1998, Lewis 2009). During this period, resistance genes from various sources were identified and characterised, and became available to cereal breeders. Australia is fortunate to have only one CCN pathotype, Ha13, allowing for effective genetic control with a few genes (Vanstone *et al.* 2008) and growers being able to deploy resistance without needing to characterise CCN populations in their fields.

Growers learnt to effectively manage CCN with resistant cultivars and rotations with pulse, oilseeds and pastures, combining this with the need to manage other soil borne pathogens. The value of estimating CCN population density as a predictive tool for risk management was established with Sironem bioassay (Brown 1987), and further developed as a soil DNA assay as part of the Root Disease Testing Service (RDTS) provided by the SA Research and Development Institute (Opehel-Keller *et al.* 2008). The on-going provision of adapted resistant cultivars is underpinned by cost-effective, high-throughput phenotyping (Lewis 2009) and application of molecular marker technology (Williams *et al.* 2003).

STATUS

The exotic nematode *H. avenae* pathotype Ha13 in Australia is widespread in southeastern Australia, but population densities have strongly declined with the deployment of resistant cultivars (Figure 2). Outlier populations in NSW and WA have not developed to more than a localised problem.

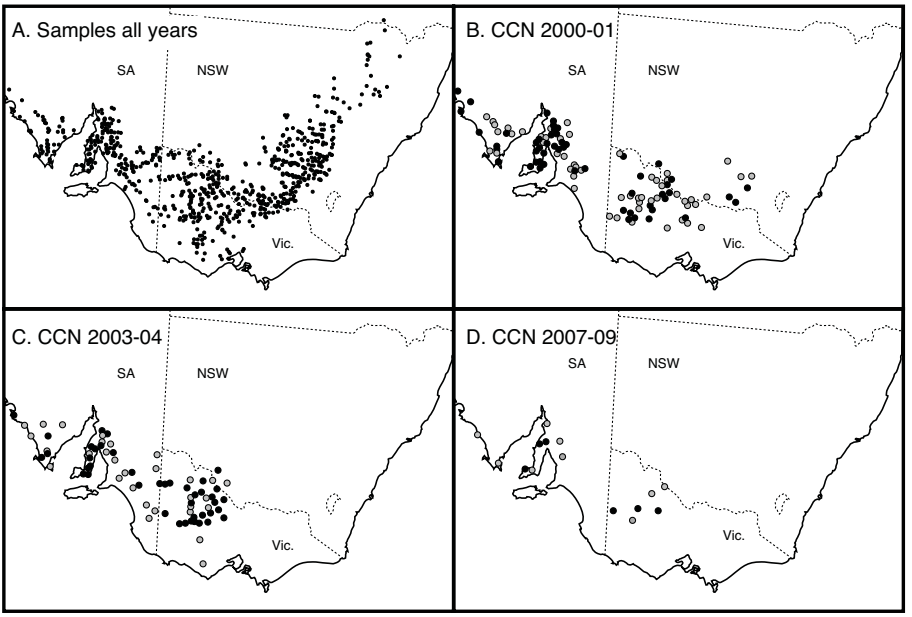

Figure 2. Cereal cyst nematode (*Heterodera avenae*) in New South Wales (NSW), South Australia (SA) and Victoria in southeastern Australia assayed in soil DNA by the SARDI Diagnostics (Ophel-Keller *et al.* 2008). A, locations from which soil was analysed, with sample numbers up to 60/location; B-D, locations in which at least one sample was positive for CCN in summer to autumn periods from 2000 to 2009, moderate to high density (closed circle), low density (open circle); B, 2000-01 (n=1785); C, 2003-04 (n=2032); D, 2007-08 and 2008-09 combined (n=1070).

The taxonomic status of CCN in Australia has been the subject of some discussion. Recently, on purely molecular grounds, the species *Heterodera australis* was proposed (Subbotin *et al.* 2002), with a suggestion of it being an Australian endemic. This proposal has not received support in Australia (Vanstone *et al.* 2008), because of the lack of morphological and ecological differences from *H. avenae* and insufficient evidence that the molecular differences justify species level distinction. CCN populations in Europe are genetically and biological variable and molecular data alone are not adequately diagnostic (Holgado *et al.* 2009). The Australian CCN is considered to have arrived as a single introduction, with subsequent spread (Mcleod 1992), which is consistent with the limited ecological (Stanton and Eyres 1994) and genetic diversity observed, and no known native hosts. Biological invaders, having established from small founder numbers passing through a genetic bottleneck, could be expected to have genotypes difficult to detect among the genetic diversity in their native range (Lockwood *et al.* 2008, Quader *et al.* 2008), which for *H. avenae* has not been fully explored.

IMPACTS

CCN in Australia was once an agricultural pest that threatened the economic viability of many farms in cropping areas of southeastern Australia. It was especially problematic because in the most heavily infested areas crops are grown in extensive, low-input, water-limited systems on relatively poor soils with high temperature and evaporative demands during grain fill; conditions that exacerbate the impact of root disease. Soil nematodes are not rapidly invasive; CCN arrived in Australia, took decades to be noticed as a pest, decades more to cause substantial impact, and then finally decline, a process not uncommon for invasive species (Davis 2009). Australian soils are too dry for the natural biocontrol seen in the UK (Kerry and Crump 1998), so the decline in Australia is a result of concerted effort of breeders and nematologists.

Although the impact of CCN in Australia has declined, this can only be maintained by on-growing breeding, effective quarantine barriers to invasion of new biotypes and surveillance for populations (introduced or evolving locally) virulent against the resistance genes currently deployed.

PROSPECTS

CCN population densities are expected to decline further unless the inevitable complacency that follows solved problems or release of highly productive susceptible cultivars results in an increase in the proportion of susceptible crops grown. CCN cannot be eradicated as some grass weeds are hosts, and it will rebound quickly if a sequence of susceptible crops are grown. Quarantine and surveillance are vital to ensure new species or biotypes do not undermine the current control.

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CURRENT KNOWLEDGE OF CEREAL CYST NEMATODE (*HETERODERA AVENAE*) ON WHEAT IN CHINA*

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SUMMARY

Survey of cereal cyst nematodes (CCN) was conducted in Chinese provinces from 2005 to 2009. Based on the morphological identification and molecular characterisation, the cysts extracted from wheat in different provinces were identified the cereal cyst nematode, *Heterodera avenae*. To date, CCN has been isolated and reported in 12 provinces at high prevalence. The population densities found were much higher than those in published reports in which CCN is recognised as economically damaging to wheat. The wheat growing area of these 12 provinces represents about 80% of the total for China, around 20 Mha, with average yields ranged from 3 to 6 t/ha depending on the agroecological region. Preliminary yield loss was determined for common Chinese wheat cultivars in three provinces including Anhui, Henan and Hebei. Treatment with the nematicide aldicarb significantly increased the yield by 10 to 40% compared to the control. These results were similar to those reported in Australia, France, India, Turkey and USA. The wide distribution of CCN in the main wheat-producing provinces of China and the

*Peng DL, Nicol JM, Li HM, Hou SY, Li HX, Chen SL, Ma P, Li HL, Riley IT (2009) Current knowledge of cereal cyst nematode (*Heterodera avenae*) on wheat in China. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 29-34. (CIMMYT: Ankara, Turkey)

magnitude of preliminary yield loss data indicate that CCN is a major biotic constraint to China's wheat production. Furthermore, morphological and molecular identification, pathotype tests, host resistance tests and yield losses have been conducted by several key institutions in China.

INTRODUCTION AND HISTORY

China is the world's largest producer of wheat, with more than 120 Mt produced per year, with average yield of about 4 t/ha. The cereal cyst nematode (CCN), *Heterodera avenae* was first reported from Hubei province in the centre of China in 1989 (Chen *et al*. 1991, 1992). It was subsequently reported in Henan (1991, 1992), Hebei, Beijing, Inner Mongolia, and Qinghai (Peng 1995), Anhui (Zheng *et.al*.1996) and Shandong (Liu *et al.* 2005), Shaanxi and Gansu (Peng *et al.* 2008) and Jiangsu (H. M. Li and D. L. Peng unpublished data). The distribution of *H. avenae* now includes 12 provinces and autonomous regions from the central, north, northwest and east of China, as shown in Figure 1. Surveys between 1998 and 2005 found CCN incidence and population density varied greatly between provinces (Table 1).

Figure 1. The distribution of cereal cyst nematode (*Heterodera avenae*) in China. Red circles indicate the surveyed provinces and blue arrows indicate the year of first detection.

In general, the CCN population densities found in China are higher than those reported from other countries where CCN is recognised as economically damaging to wheat. The total wheat production area of these 12 provinces is about 20 Mha and represents about 80% of China's total wheat production area, with average yields ranging from 3 to 6 t/ha depending on the agroecological region. In Henan, which is the largest wheat producing province in China, CCN infests to more than 1.2 Mha.

Table 1. Range in *Heterodera avenae* incidence and population densities in determined in surveys between 1998 and 2005 in China.

Province/City	Samples (no)	Proportion infested (%)	Population range (eggs/g soil)
Beijing	124	64	36-96
Hebei	108	93	21-150
Henan	22	73	9-12
Hubei	126	99	1-45
Inner Mongolia	5	100	70-90
Qinghai	120	100	3-75
Shanxi	2	100	51

The distribution of *H. avenae* in the northeast provinces of China was unknown because no systematic survey of CCN had undertaken in this region (Peng *et al.* 2007, Riley *et al.* 2007).

The preliminary yield losses trials were conducted for common Chinese wheat cultivars in three key provinces, Henan, Hebei and Anhui. Treatment with the nematicide aldicarb significantly increased the wheat yield by 10 to 40% compared to untreated wheat. However, total yield loss has been observed in winter wheat in some particularly heavily infested fields (Peng *et al.* 2007).

The yield losses from *H. avenae* infestation of wheat in northern China are comparable to those reported in Australia, France, India, Turkey (J. M. Nicol unpublished data) and USA. The wide distribution, high incidence and non-specific symptoms on wheat caused by CCN in key wheat producing provinces of China has have raised concerns about the potential impact of this nematode pest. It is now considered that CCN is a major biotic constraint to wheat production in China.

The pathotypes of four CCN populations from wheat fields of four provinces in China (Dinzhou, Hebei; Fangshan, Beijing; Xinxiang, Henan; Tianmen, Hubei) were recently tested by the international standard host differential developed by Andersen and Andersen (1982). Apparently at least three pathotypes were revealed by these tests and where different from other known pathotypes. None of these three pathotypes reproduce on any of the six oat differentials. Only a few of the 12 barley differentials were susceptible. The three pathotypes showed different responses from each other in wheat cultivars, but similar to the results reported in other parts of the world. None of the six wheat differentials or three local wheat cultivars included were resistant to all three pathotypes. The reactions of these three pathotypes differed from those of previously described by Peng and Cook (1996). Likewise, Yuan *et al.* (2009) have also reported recently two new pathotypes from Zhengzhou, Henan, with wider virulence than those described by Peng and Cook.

Morphological and molecular characterisation of the selected samples revealed a close relationship between species within the *H. avenae* group. A fragment of approximately 1060 bp was amplified from the rDNA-ITS region of *H. avenae* from China and Morocco with primers AB28 and TW81 (Joyce *et al.* 1994). Restriction fragment length polymorphism of the ITS regions (ITS-RFLP) within the ribosomal

DNA (Peng *et al*. 2003, Ou *et al*. 2008) classified the Chinese CCN populations as "type B" according to Subbotin *et al*. (1999).

With the recognition of the importance of CCN in China, a Chinese CCN network was formed and work initiated in collaboration with CIMMYT and Australia (SARDI). Many aspects of the ongoing and future research of CCN in China will be discussed in the recently formed International Cereal Cyst Nematode Initiative.

STATUS AND PROSPECTS

H. avenae is distributed across many wheat producing regions of the north and northwest China. It occurs in irrigated and rain-fed fields where the mean annual precipitations range from 300 to 1200 mm. Little effort has been made to prevent the further spread of *H. avenae*. However, it is recognised that uninfested fields can be protected by farm hygiene practices which limit the chance of introducing soil from infested areas.

As in other parts of the world, the impact of *H. avenae* in China could be reduced the decreasing the frequency of host crops (wheat, barley and oat) in the rotation and increasing the frequency of broadleaf crops such as rapeseed, and maize (D. L. Peng unpublished data). The rotation of broadleaf crops is generally an economical and viable option for the driest areas of the northwest wheat-growing belt in Qinghai province.

In the USA, nematicides are not registered for application in wheat and no biocontrol agents or seed treatments have proven effective under field conditions (Smiley *et al.* 2007). Therefore, management of *H. avenae* in the western USA has been accomplished most efficiently through genetic resistance, with an initial focus on the *Cre1* gene (Smiley *et al.* 2007). Likewise in China, no nematicides are registered for application in wheat. Therefore, management of *H. avenae* in the northern China will be most likely be accomplished through genetic resistance, although the resistance and/or tolerance status of most Chinese wheat germplasm is currently unknown.

IMPACTS

Estimates of total area infested with potentially damaging populations of *H. avenae,* yield reduction and economic losses from CCN in three province in north China are given in Table 2. The area known to be infested with potentially damaging population densities represents about 22% of the total wheat production area. Therefore, it is estimated that the overall yield suppression in the infested areas is about 10%. Wheat yield reduction is estimated at 1.2 Mt; valued at 1.9 billion RMB.

It is also recognised that wheat growers and agricultural advisors need more information on CCN to improve awareness and increase their ability to respond.

Table 2. Estimation of the impact of *Heterodera avenae* on wheat production and profitability in three key provinces of China.

	Henan	Hebei	Anhui	Total
Wheat production				
Wheat growing area (1000 ha)	5,260	2,440	2,410	10,110
Wheat yield (t/ha)	5.79	5.03	4.91	5.24
Production (Mt)	30.5	12.3	11.9	54.7
Value (billion RMB)	48.8	19.7	19.0	87.4
Impact of *Heterodera avenae*				
Infested wheat area (%)	23	21	22	22
Yield reduction (%)	10	10	10	10
Reduced production (Mt)	680	258	260	1,198
Total loss value (billion RMB)	1.09	0.41	0.42	1.92

RECOMMENDATIONS

At present, the biology, ecology and genetic structure of Chinese populations are CCN have not been adequately investigated and are poorly understood compared to comparable situations in Australia, Europe and the USA. It is recommended that CCN from north and northwest China be more thoroughly investigated and included in international comparative studies. The reactions of Chinese wheat germplasm to local CCN populations needs to be characterised as a matter of priority.

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OCCURRENCE, DISTRIBUTION AND CONTROL OF *HETERODERA AVENAE* AND *H. FILIPJEVI* IN THE WESTERN USA*

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SUMMARY

Heterodera avenae occurs in at least seven states in the western USA. *Heterodera filipjevi* was first reported in North America during 2008 and is currently reported only in Oregon, where it occurs as a mixture with *H. avenae*. Juveniles of both species emerge from cysts primarily during the spring. It is estimated that *H. avenae* reduces wheat yield by 21,000 t, valued at US$3.4 million, in the Pacific Northwest states of Idaho, Oregon and Washington. Neither *H. avenae* nor *H. filipjevi* pathotypes in the Pacific Northwest are adequately characterised by indexing plants of the current International Test Assortment. However, reproduction of *H. avenae* is consistently absent in wheat containing the *Cre1* gene and in barley containing the *Rha2* gene. A donor of the *Cre1* gene was crossed with locally-adapted wheat cultivars and the crosses are being screened for resistance. Rotation of winter wheat with weed-free broadleaf crops or long fallow (14 months) reduces damage to subsequent wheat but rotations often are not profitable in the driest areas of the region. Nematicides are not registered for managing damage by cereal cyst nematodes in North America. Fungal parasites of cysts and/or eggs have been detected but have not been investigated and do not appear to provide effective control in Oregon.

INTRODUCTION AND HISTORY

Heterodera avenae in North America was first reported in the Province of Ontario, Canada (Chapman 1938). Three decades later this species was present in most counties of that province (Fushtey 1966). The first detection of *H. avenae* in the United States was in Oregon (Jensen *et al*. 1975). It was subsequently reported in California (Hackney 1981), Washington (Hafez and Golden 1984), Michigan (Graney 1985), Idaho (Hafez and Golden 1985) and in additional areas of Oregon

*Smiley RW (2009) Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in western USA. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 35-40. (CIMMYT: Ankara, Turkey)

(Hafez *et al*. 1992, Smiley *et al*. 1994, Smiley *et al*. 2007). The distribution of *H. avenae* in the western USA now includes selected areas within the states of California, Colorado, Idaho, Montana, Oregon, Utah and Washington (Figure 1; Smiley *et al*. 2007). *H. filipjevi* was first reported in North America during 2008 (Smiley *et al*. 2008, Yan *et al*. 2008, Yan and Smiley 2009). The extent to which *H. avenae* and *H. filipjevi* are actually distributed in the western USA remains unknown because there have been no systematic surveys of these species in these regions.

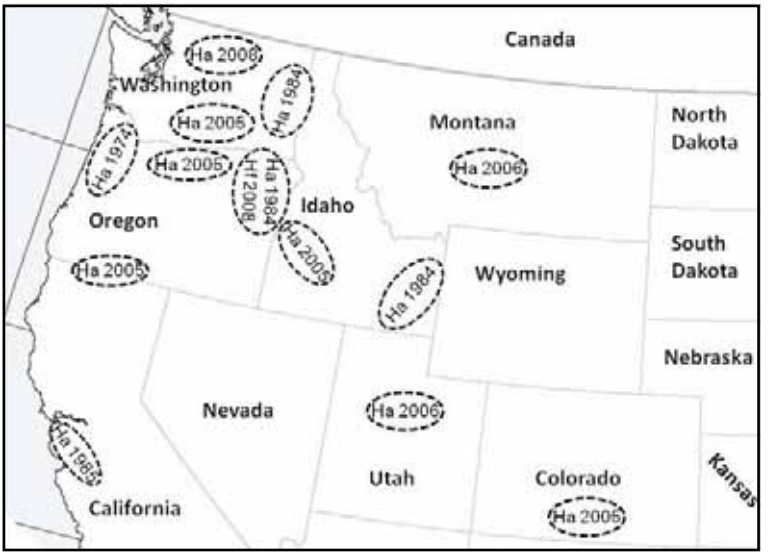

Figure 1. Location and year in which infestations of *Heterodera avenae* (Ha) and *Heterodera filipjevi* (Hf) were publicly reported in the western USA.

Crop damage assessments for *H. avenae* on wheat in Oregon were reported by Smiley *et al*. (1994, 2005). Spring and winter wheat yields in field trials were reduced as much as 25 and 50%, respectively, but it was also observed that stands of spring wheat were totally destroyed in at least some highly-infested commercial fields. Yield losses from *H. avenae* on wheat in Oregon are comparable to those reported elsewhere in the world.

Peak populations of *H. avenae* and *H. filipjevi* juveniles in Oregon occur primarily during the spring (Smiley *et al*. 2005, G. P. Yan, R. W. Smiley and H. Yan unpublished data), similar to the northern ecotype described by Rivoal (1986).

The single-dominant *Cre1* gene effectively halts reproduction of four *H. avenae* populations collected from western Oregon, eastern Oregon, southeast Idaho and eastern Washington, as illustrated by results of a recent test with selected entries of the International Test Assortment (Table 1). Cultivars containing the *Cre1* gene, such as AUS10894, Loros (63/1.7.15.12) and Ouyen have consistently been very effective against these populations in screenings performed in the glasshouse and outdoor nursery. Ouyen is an Australian hard-white spring wheat cultivar with good agronomic traits and was used as the *Cre1* gene-donor parent for crossings with spring and winter wheat cultivars adapted to the Pacific Northwest environment. Many of these crosses have been shown to be resistant to *H. avenae* in preliminary

tests and are being evaluated further before being tested in naturally-infested fields. While Iskamish-K2-light exhibited resistance to each of the three soils in these tests it was rated from susceptible to resistant in earlier tests with other soils from eastern Oregon, suggesting the need for further testing to determine if multiple pathotypes may be present in the region. Wheat lines carrying the *Cre3*, *Cre7* and *Cre8* genes are susceptible to eastern Oregon populations of *H. avenae*, and lines with *Cre2* and *Cre5* are moderately resistant but variable across tests. Nevertheless, it is possible that benefits from *Cre5* may already occur across the region because the *Cre5* gene is carried by the French cultivar VPM1 which, along with many of its derivatives, has served as the source of eyespot resistance in many Pacific Northwest cultivars.

Table 1. Resistance[1] of selected entries of the International Test Assortment to populations of *Heterodera avenae* from southeast Idaho, eastern Oregon and eastern Washington.

Entry	Gene[2]	Idaho	Oregon	Washington	Mean
Barley					
Bajo Aragon 1-1	*Rha2*	0	0	1	0
Herta	-	2	5	31	13
Ortolan	*Rha1*	4	2	15	7
Oat					
Nidar II	-	15	24	60	33
Silva	-	5	2	15	7
Sun II	-	8	8	34	17
Wheat					
AUS 10894	*Cre1*	0	0	0	0
Capa	-	6	5	33	15
Iskamish-K2-light	?	0	0	1	0

[1]Mean number of white cysts (mean of 2 experiments with 7 replicates each) produced on individual plants grown during the spring in an open-bottom pot of naturally-infested soil (250 g), from which roots grew into an irrigated and fertilised bed of sand. Pre-plant populations of eggs plus juveniles from cysts were 52800, 3000 and 9760/kg of Idaho, Oregon and Washington soil, respectively. [2]Where present and known, the identity of the resistance gene is shown.

In a test in which *H. filipjevi* from eastern Oregon was screened for resistance using the International Test Assortment plus additional entries we observed reproduction at low rates in plants carrying the *Cre1* gene. This preliminary result will be re-evaluated to determine if additional sources of resistance are required where *H. avenae* and *H. filipjevi* occur in mixed populations. If so, emphasis will be given to accessions that prevented reproduction of *H. filipjevi* in the initial screening.

Resistances to Oregon populations of *H. avenae* were also detected in barley lines carrying the *Rha2* gene in Bajo Aragon 1-1, KVL191 and Martin 403-2, and the

Rha3 gene in Morocco. Likewise, resistances to the Oregon populations were detected in the oat accessions Pusa Hybrid (640318-40-2-1), Silva (KVL1414), I376 (CC4658) and IGVH72-646 (MK.H.72-646). These potential donor parents could prove useful where these crops have been damaged by *H. avenae*, such as oats in western Oregon and malting barley in Colorado.

STATUS AND PROSPECTS

H. avenae is distributed across many small grain producing regions of the western United States. It occurs in rain-fed and irrigated fields in wheat producing regions having a mean annual precipitations ranging from 250 to 1000 mm. Essentially nothing is known about the distribution of *H. filipjevi*. However, with little effort being expended to prevent further dissemination of *H. avenae*, it is assumed that *H. filipjevi* is also being disseminated. However, it is well recognised that uninfested fields can be protected from becoming infested by sanitary practices that prevent infested soil from being introduced into "clean" land.

As in other parts of the world, damage from *H. avenae* in Oregon can be controlled by reducing the frequency of host crops in the rotation, such as rotating wheat, barley or oats with a broadleaf crop or a weed-free summer fallow (Smiley *et al*. 1994, Smiley and Nicol 2009). However, rotations in the Pacific Northwest are economical mostly in irrigated crops and in dryland crops produced in high rainfall districts. Broadleaf rotation crops are generally not an economically viable option in the driest areas of the wheat belt of semiarid eastern Oregon and Washington.

Nematicides are not registered for application to wheat in the United States and there are no biological agents or seed treatments known to be effective under field conditions. Management of *H. avenae* in the western USA is will be accomplished most efficiently through genetic resistance, with an initial focus on the *Cre1* gene.

Molecular markers for the *Cre1* gene have been developed in other countries and will be an important tool in marker-assisted selection procedures for increasing the efficiency of wheat breeding programs. However, current markers are proprietary and are not readily available to other scientists. Development of a *Cre1* marker for use in North America is underway. Likewise, a molecular diagnostic test has been developed to differentiate *H. avenae* and *H. filipjevi* quickly and at little expense (Yan *et al*. 2009, Yan and Smiley 2010).

IMPACTS

Table 2 provides an estimate of land infested with potentially damaging populations of *H. avenae* and of yield reduction and economic losses from this species in the Pacific Northwest. While areas known to be infested with potentially damaging population densities represent only 0.04% of the production area, it is estimated that the overall yield suppression in the infested areas is about 10%. Reduction of wheat yield in the region is therefore estimated at 21,000 t valued at US$3.4 million.

Further education of farmers and their commercial and public-sector advisors is required to increase the level of awareness of cereal cyst nematodes. Most growers and advisory personnel in the western USA do not readily differentiate patches of

depressed growth caused by cereal cyst nematodes, fungal pathogens such as *Gaeumannomyces graminis* and *Rhizoctonia solani*, and edaphic factors associated with non-uniformity of the soil substrate. For example, the growers who donated soil for studies shown in Table 1 only recently became aware that cereal cyst nematodes were the primary cause of depressed patches of wheat in their fields.

Table 2. Estimate of the impact of *Heterodera avenae* on wheat production and profitability in three Pacific Northwest states.

Wheat Statistics	Oregon	Washington	Idaho	Pacific Northwest
All wheat[1]				
area (1000 ha)	855	2,137	1,175	4,167
yield (t/ha)	3.62	3.99	4.71	4.11
production (Mt)	1.25	3.45	2.24	6.95
value[2] (million US$)	210	578	348	1,135
Impact of *H. avenae*				
area infested (%)	0.05	0.01	0.05	0.04
yield reduction in infested area (%)	0.10	0.10	0.10	0.1
reduced production (t)	6,269	3,449	11,214	20,933
total value (thousand US$)	1,047	576	1,738	3,361

[1]Data from the US National Agricultural Statistics Service for 2007. [2]Value was based upon a mean farm-gate income of 167, 167, 155 and 163 US$/ton for Oregon, Washington, Idaho and the Pacific Northwest, respectively. Wheat is produced under rain-fed or irrigated conditions in low- to intermediate-rainfall areas, with 90, 90, 60 and 80% of the harvested area being rain-fed in Oregon, Washington, Idaho and the Pacific Northwest, respectively.

RECOMMENDATIONS

North American populations have been generally absent or of minor interest in comparative global investigations of the biology of the *H. avenae* group. Future investigations of cereal cyst nematode biology, nationally and internationally, would benefit from the inclusion of populations from the western USA.

ACKNOWLEDGMENTS

Funding was from the United States Department of Agriculture-Agriculture Research Service and the Idaho Wheat Commission, Oregon Wheat Commission and Washington Wheat Commission. Land and soil were donated by wheat producers in Idaho, Oregon and Washington. Technical services were provided by Drs W. Uddin and J. A. Biddle, and by G. H. Cook, S. A. Easley, J. A. Gourlie, L.-M. Patterson, J. G. Sheedy, R. G. Whittaker and H. Yan. Scientific advice and assistance were provided by Drs Z. A. Handoo, R. E. Ingham, H. Kreeft, J. M. Nicol, T. C. Paulitz, J. N. Pinkerton, T. O. Powers, R. Rivoal, J. M. Windes and G. P. Yan. Dr G. P. Yan (Oregon State University, Pendleton) is thanked for reviewing this paper before submission.

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GLOBAL IMPORTANCE OF THE MAIN NEMATODES ASSOCIATED WITH CEREALS IN TUNISIA*

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SUMMARY

The survey carried out in the main production cereal areas in Tunisia showed nematode complex infestation frequencies of 68, 89 and 76% respectively in the sub-humid, semi-arid and upper arid bioclimates. The main nematode populations belonged to the genera *Heterodera* and *Pratylenchus*, which co-occurred in 60% of fields. There appeared to be competition between these two genera with nematode infestation rate greater for *Heterodera* than *Pratylenchus*. *H. avenae* was the most common cereal cyst nematode species in Tunisia and the common root lesion nematodes were *P. thornei, P. neglectus* and *P. penetrans*. Assessment of *H. avenae* damage on the different yield components of wheat (cv. Karim) and barley (cv. Rihane) and of nematode multiplication rate (Rf) was performed in experiments under field conditions. Multiplication rates were inversely correlated with initial nematode densities. *H. avenae* reproduced readily on both wheat and barley cultivars with Rf ranging from 4 to 8 in wheat and 5 to 11 in barley. Synchronisation between hatching of *H. avenae* and the sowing period of wheat and barley under Mediterranean conditions is likely to result in heavy early infestation and crop losses.

INTRODUCTION

In Tunisia, nematodes are becoming of a great importance in cereals, with *Heterodera* and *Pratylenchus* species likely to have the greatest impact. Although *Pratylenchus* species are cosmopolitan and polyphagous, the cereal cyst nematode (CCN), *Heterodera avenae* has been reported as the most damaging nematode to wheat and barley around the world (Rivoal and Cook 1993).

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*Namouchi-Kachouri N, B'Chir MM, Hajji A (2009) Global importance of the main nematodes associated with cereals in Tunisia. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 41-44. (CIMMYT: Ankara, Turkey)

The relationship between initial population density (Pi) of CCN and yield of wheat and barley is important in determining the nematode's economic impact on these crops. Yield losses have been reviewed by Nicol (2002) and ranged from 15-20% in wheat in Pakistan, 40-90% in wheat and 17-77% in barley in Saudi Arabia and 20% in barley and 23-50% in wheat in Australia. Reductions of wheat and barley yields by *H. avenae* have also been reported from the Mediterranean climate regions of Libya, Morocco and Italy.

The aims of this work were to identify the relationship between the main nematode species and to assess the effect of Pi of *H. avenae* on wheat and barley yield and on nematode reproduction under field conditions in Tunisia.

METHODS

Soil and root samples were collected from around 200 cereal fields in the major cereal growing areas. The survey being initiated to identify the main nematode species, their prevalence and associations.

In addition, two adjacent fields (40 ha each) in the cereal-producing area of Zaghouan, an area of semi-arid climate, were selected for detailed study. One field was to be sown to wheat (*Triticum durum* cv. Karim) and the other barley (*Hordeum vulgare* cv. Rihane), and both were naturally infested with *H. avenae*. At sowing, sites with varying Pi were sampled and plots were then selected to give a range of Pi (Table 1). Five replicate plots were assigned for each Pi. Plots free of nematodes were used as controls and the reproduction factors (Rf) were calculated.

At harvest, all plants within each plot were removed by hand and data on yield components (Table 1) were recorded. Data from each treatment were analysed by ANOVA (Fisher's protected LSD). Regression analyses were also performed to describe the relationship between Pi and grain yield, Pf and Rf, and between co-occurring *Heterodera* and *Pratylenchus* populations (STATISTICA 5.0).

RESULTS

Field infestation

The field infestation frequencies were 68, 89 and 76% in the sub-humid, semi-arid and upper arid climatic regions, respectively. The main nematode genera present were *Pratylenchus* and *Heterodera*, which co-occurred in 60% of infested fields. Non-linear regression analysis showed an inter-specific competition between the two genera (Figure 1).

Population change and yield impact

Results from both experiments were very similar. As Pi increased, *H. avenae* suppressed ($P \leq 0.05$) all yield components in wheat and barley (Table 1). Grain yields were reduced by 19 to 86% in barley and 26 to 96% in wheat. The suppression of these parameters, as well as final population densities (Pf), increased ($P \leq 0.05$) with increasing of Pi, whereas Rf decreased.

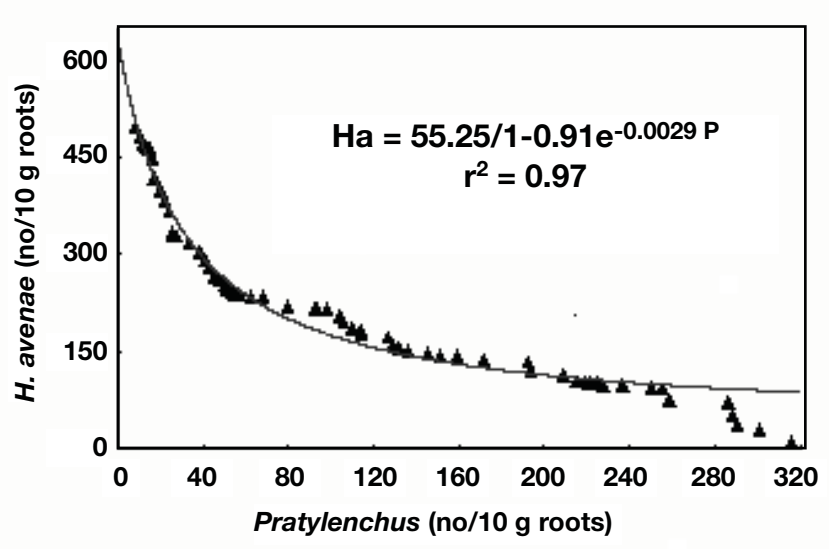

Figure 1. Relationship between *Heterodera* (Ha) and *Pratylenchus* (P) population densities (nematodes/10 g of roots) in concomitant populations in Tunisia.

Table 1. Effects[1] of initial density of *Heterodera avenae* on its reproduction and yield of wheat (cv. Karim) and barley (cv. Rihane) under field conditions in Tunisia.

Pi[2]	Kernels/ spike	Spikes/m²	1000 kernel weight (g)	Yield (g/m²)	Yield reduction (%)	Pf[3]	Rf[4]
Wheat							
0	31 a	246 a	43 a	329 a	-	-	-
1060	29 a	228 a	40 a	267 b	26	9190 a	8.67 a
2530	23 b	190 b	35 b	154 c	64	15310 b	6.05 b
4510	12 c	145 c	25 c	44 d	96	19120 c	4.60 c
Barley							
0	28 a	212 a	32 a	196 a	-	-	-
1420	26 a	195 a	31 a	157 b	19	15650 a	11.02 a
3280	21 b	161 b	27 b	91 c	53	22115 b	6.74 b
4870	12 c	118 c	20 c	28 d	86	26150 c	5.37 c

[1]Data followed by the same letters are not significantly different (P≤ 0.05). [2]Pi = number of second stage juveniles (J2) plus encysted eggs/100 g soil at planting. [3]Pf = number of J2 plus encysted eggs/100 g soil at harvest. [4]Rf = Pf/Pi.

Regression analysis showed that grain yield of wheat was negatively correlated with Pi ($y=313-0.07 \cdot Pi$, $r^2=0.87$, $P \leq 0.001$) and similarly for barley ($y=184-0.04 \cdot Pi$, $r^2=0.86$, $P \leq 0.001$). Pf was positively correlated with Pi in wheat ($y=6796+3.7 \cdot Pi$, $r^2=0.81$, $P \leq 0.001$) and barley ($y=11428+3.3 \cdot Pi$, $r^2=0.76$, $P \leq 0.001$). However, Rf was negatively correlated with Pi in wheat ($y=10.3-0.002 \cdot Pi$, $r^2=0.75$, $P \leq 0.001$) and barley ($y=13.2-0.002 \cdot Pi$, $r^2=0.77$, $P \leq 0.001$).

DISCUSSION

The relationships between *Heterodera* and *Pratylenchus* are complex and subject to the effects of many factors: kind of parasitism, pathogenicity and environmental factors (Eisenback 1985, Khan and Khan 1990). Migratory endoparasitic nematodes infect host plants more rapidly and inhibit the penetration of sedentary species (Gay and Bird 1973). In other cases, because of complex way they alter host physiology (e.g. formation of syncytia), sedentary endoparasitic nematodes may inhibit the development of *Pratylenchus* and affect their reproduction (Gay and Bird, 1973).

H. avenae reproduced readily in both wheat and barley cultivars and Pf increased as Pi increased, with Rf ranging from 4.6 to 11. Generally, the multiplication rate was higher in barley than in wheat, which supports the findings of CCN infestations (Wolny 1990). Rf were negatively correlated with Pi in both plants, as has been previously reported (Magi 1989). This could be attributed to competition for feeding sites, nematode establishment in roots and reproduction. The impact on wheat and barley yields caused by *H. avenae* in this study are consistent with reports from different parts of the word (Nicol 2002), and show that *H. avenae* is a real threat to Tunisia cereal crops. Furthermore, synchronisation between hatching of *H. avenae* eggs and sowing period of wheat and barley under Mediterranean climatic conditions is likely to result in heavy early infestations and crop losses (Rivoal 1982).

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IMPORTANCE AND DISTRIBUTION OF THE MAIN CEREAL NEMATODES IN MOROCCO*

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SUMMARY

A survey on cereal crops (wheat, barley and oat) was carried out in 2007 in several regions of Morocco. It revealed, for the first time, the presence of the root lesion nematode *Pratylenchus* spp. in most areas surveyed. Two species of *Pratylenchus* (*P. thornei* and *P. penetrans*) were identified in the regions of Gharb, Sais, Zâer and Tadla. The percentages of infested samples was 69, 54, 50 and 40% in soil, and 63, 68, 70 and 60% at roots in Gharb, Zâer, Sais and Tadla, respectively. The cereal cyst nematode, *Heterodera avenae,* was found only in the Gharb (Sidi Slimane region). Stem nematode, *Ditylenchus dipsaci* was found in most areas surveyed, with more than 20% of samples containing this nematode. The percentage of infested samples varied from 5% in the Tadla to 34% in Gharb.

INTRODUCTION

Cereal nematodes, in particularly *Pratylenchus* spp. and *Heterodera avenae* are widely spread throughout the world and cause large yield losses crops of a range of cereal species (Caubel *et al.* 1980, Mokabli *et al.* 2001).

Whitehead (1998) reported that 10% of the worldwide production of cereals are lost because of plant-feeding nematodes. In Europe, more than 50% of cereal fields are infested by *H. avenae*, which causes an economically important damage (Rivoal *et al.* 1986).

In Morocco, the presence of *H. avenae* was reported for the first time by Franklin in 1951 (Ritter 1982, Meskine and Abbad Andaloussi 1984). Other surveys of various cereal production regions found *H. avenae* in wheat and barley in the regions of Abda, Chaouia, Doukkala, Gharb, Sais, Souss, Tadla, Zemmour-Zâers and Haouz (Himmich 1987, Sbaihi 2003, Znasni 2004).

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*Mokrini F, Abbad Andalouss F, Alaoui Y, Troccoli A (2009) Importance and distribution of the main cereal nematodes in Morocco. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 45-50. (CIMMYT: Ankara, Turkey)

Also root lesion nematode, *Pratylenchus* spp., has been found also in several cereal production regions, including Chaouia, Doukkala, Sais, Souss, Tadla, Zâers and Rhamna (Himmich 1987, Meskine and Abbad Andaloussi 1984, Sbaihi 2003). Other surveys in Morocco found confirmed *Pratylenchus thornei*, *P. penetrans* and *P. neglectus* in the regions of Sais, Doukkala and Gharb (Meskine and Abbad Andaloussi 1984).

The objective of this survey was to complete the available data on the distribution of cereal nematodes in Morocco by surveying additional regions and to determine potential impact in the main cereal crops.

METHODS

The survey concerned the main cereal crops: tender wheat, the hard wheat, oat and barley. Sampling sites of those crops are the following: Gharb, Sais, Tadla and Zâer.

According to the cereal crop importance in the region, plant sampling has been done in randomly chosen sites all 5 to 15 km. At the site level, the sampling has been achieved at the level of a certain number of 1 m² plots. For the quality of sampling, these plots have been repeated each randomly in an area of 10 m² according to the surface of the field while browsing the parcel in crisscross.

Samples consisted of both root and soil taken from plants showing poor growth and/or symptoms of leaf yellowing. These samples are put in plastic bag then brought back to the laboratory for analysis. The extraction of nematodes from soil sample has been made according to the technique of Baermann, contrarily for root sample, the extraction of nematodes has been made according to the technique of double centrifugation.

RESULTS AND DISCUSSION

The genera and species of plant-feeding nematodes found in association with cereals in the regions surveyed in Morocco are shown in Table 1. The nematode fauna associated to cereal crops consisted of 8 genera (Table 1). Of these the genera known to cause economic damage are *Heterodera* sp., *Pratylenchus* spp. and *Ditylenchus dipsaci*. These nematodes cause important damages on cereals in many parts of the world (Whitehead 1998). *Pratylenchus* spp., *D. dipsaci* and *Tylenchorhynchus* sp. were the nematodes that occurred at the highest frequency across all regions, occurring at 66.5, 23 and 4.6%, respectively in a total of 116 samples (Table 2). These results confirm those found previously by Meskine and Abbad Andaloussi (1984) and show that *Pratylenchus* spp. are widely distributed in Morocco, followed by *D. dipsaci*. In contrast, the *H. avenae* was found only in the Gharb (a region of Sidi Slimane).

The frequency of these eight types of nematode varied been the different crops sampled (Table 3). *Pratylenchus* was found in all crop species, most commonly in bread wheat and least in durum wheat. Symptoms of *Pratylenchus* infestation appear were patches in the field with a pale foliage; reddening of leaf tips and overall yellowing, stunting of the plants and a serious reduction in tillering. Two species of root lesion nematodes, *P. penetrans* and *P. thornei,* were found in the cereal root zone. Losses of wheat yield attributed to *P. thornei* have been estimated at 38 to

Table 1. Plant-feeding nematodes found in association with cereals in four regions of Morocco.

Nematode	Gharb	Tadla	Sais	Zâer
Aphelenchus	X			
Ditylenchus dipsaci	X	X	X	X
Heterodera	X			
Hoplolaimus	X		X	
Meloidogyne	X			
Pratylenchus spp.	X	X	X	X
P. penetrans	X	X	X	X
P. thornei	X	X	X	X
Tylenchorhynchus	X			X
Tylenchus	X			X

Table 2. Frequency of detection of plant-feeding nematodes in soil and plant samples collected in a survey of cereal fields in Morocco.

Nematode	Infested soil samples (%)	Infested root samples (%)
Pratylenchus spp.	66.5	51
Ditylenchus dipsaci	23	1.4
Tylenchorhynchus	4.6	
Hoplolaimus	3	
Tylenchus	3	
Heterodera	1.5	
Meloidogyne	0.8	
Aphelenchus	0.8	

Table 3. Frequencies of plant-feeding nematodes found in soil form major cereal crop species grown in Morocco.

Nematode	Cereal crops			
	Bread wheat (soft)	Durum wheat	Barley	Oat
Ditylenchus dipsaci	13	0	1	2
Pratylenchus spp.	41	6	22	10
Heterodera avenae	2	0	0	0
Meloidogyne spp.	2	0	0	0
Tylenchorhynchus	2	1	1	4
Hoplolaimus	0	0	0	1

85% in Australia (Nicol 1996, Taylor *et al.* 1999) and 32 to 70% in Israel (Orion 1984).

Root lesion nematodes constitute a real threat to cereal production in Morocco. Indeed, results our showed that these nematodes are very widespread across the regions sampled (Figure 1). These nematodes have a wide host range, including the legumes grown in rotation with cereals in Morocco.

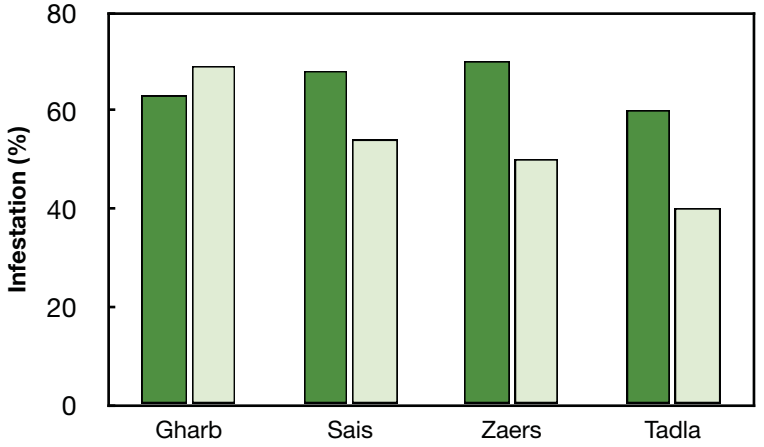

Figure 1. Per cent *Pratylenchus* infestation of soil (dark bars) and plant (light bars) sample from four cereal regions of Morocco.

The highest density or *Pratylenchus* infestation was found in oat roots (over 2000 per 100 ml; Table 4). The other crops, bread wheat, durum wheat and barley had about 10-fold less (Table 4).

Table 4. Average concentration *Pratylenchus* (no/100 ml) in soil and root samples from the major cereal crops sampled in Morocco.

Crop	Soil	Root
Bread wheat (soft)	43	200
Durum wheat	19	80
Oat	126	2520
Barley	14	200

Stem nematode, *D. dipsaci,* was found at variable frequency in the cereal species sampled, being most found in bread wheat (Table 3) and in 31, 27, 24 and 5% of samples from Gharb, Sais, Zâer and Tadla, respectively (Figure 2**)**. In the root zone, the highest frequency of 34% was recorded in the Gharb. Previously, *D. dipsaci* was found in oat in Morocco at a frequency of 31% and densities of 24 nematodes/g dry matter (Abbad Andaloussi 1996). The symptoms of infestation by *D. dipsaci* in oat are dwarfing, abundant tillering and leaf distortion (Abbad Andaloussi 1996).

According to these preliminary results, it appears that *Pratylenchus* spp. and *Ditylenchus dipsaci* are the most common nematode pests of cereals in the regions

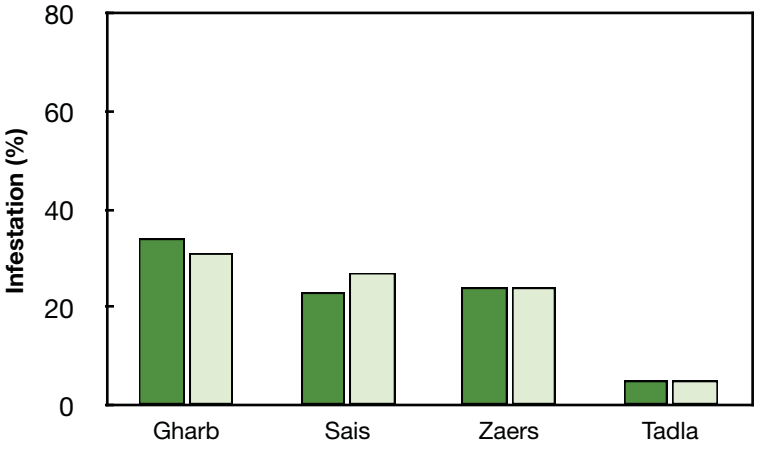

Figure 2. Per cent *Ditylenchus dipsaci* infestation of soil (solid bars) and plant (open bars) sample from four cereal regions of Morocco.

surveyed. Two species of *Pratylenchus* spp. were identified and are highly abundant. These *Pratylenchus* spp. are characterised by wide host range, which includes important crop legumes, so can particularly affect wheat and barley yield when these legumes are grown in rotation with cereals. *H. avenae* was found exclusively in Gharb region (at a site in Sidi Slimane). Therefore, avoid accelerating the spread of *H. avenae* to uninfested regions, appropriate farm hygiene and other phytosanitary method should be adopted.

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CEREAL CYST NEMATODES, ROOT ROT PATHOGENS AND ROOT LESION NEMATODES AFFECTING CEREAL PRODUCTION IN IRAN*

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SUMMARY

In Iran, the distribution of cereal cyst nematodes and root lesion nematodes was surveyed, with soil borne pathogenic fungi and soil properties being characterised in some samples. In total, 425 soil and root samples were collected, mainly from rain-fed cereal fields in the west, north and central west, north regions. The cereal cyst nematodes (CCN), *Heterodera filipjevi*, *H. latipons* and *H. avenae,* were found in 34% of soil samples (ranging from 18 to 50% between regions). *H. filipjevi* was the most common and widely distributed of the three species. Population densities of CCN in some soil samples were more than 50 cysts/300 g soil, possibly more than the critical threshold for damage. The root lesion nematodes (RLN), *P. thornei* and *P. neglectus,* were found in 13 and 40% of soil samples, respectively, and *Pratylenchoides ritteri* was present in 6% of soil samples mostly in the western regions. Root rot pathogens found in some plant material, including *Fusarium pseudograminearum, F. culmorum, Bipolaris sorkokinana* and *Rhizoctonia cerealis,* as causal agents of crown and root rot. These were frequently found in combination with the RLN and/or CCN.

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INTRODUCTION

Soil borne nematodes in cereal cultivation mostly belong to two groups of plant parasites, cereal cyst nematodes (CCN) and root lesion nematodes (RLN). These are distributed globally and implicated in significant economic yield loss. Root rots generally include a complex of species such as common root rot (*Bipolaris sorokiniana*) and several species of crown root (*Fusarium* spp.). Yield loss caused by these cereal root pathogens have been documented in many regions of the world including Europe, North America and particularly in the more marginal cereal production areas of west Asia, north Africa, Australia and Canada (Nicol *et al*. 2004, Smiley *et al*. 2005a,b). The two most commonly reported *Fusarium* species are *F. pseudograminearum* and *F. culmorum*, with several others, such as *F. acuminatum*, *F. avenaceum* and *F. crookwellense*, being reported less frequently.

Five species of cyst-forming nematodes belonging to the *Heterodera avenae* group were identified from cereal fields and grasslands in Iran, of which *H. filipjevi* and *H. latipons* are the prevalent species in cereal fields (Tanha Maafi *et al*. 2007). Root lesion nematodes, *Pratylenchus neglectus* and *P. thornei,* have frequently been reported from cereal fields in many provinces of Iran (Kheiri 1972). Also, root rot pathogenic fungi have been isolated from several wheat production regions in Iran (Ravanlou and Banihashemi 1999, Heidarian and Ershad 2001, Kazemi 2002). The objective of this survey was to determine the distribution and population density of cereal nematodes, along with root rot pathogenic fungi and soil properties of some samples, particularly from rain-fed wheat growing fields.

METHODS

Four hundred and twenty five soil and root samples and 45 plant samples were collected from various regions of Hamedan, Kermanshah, Markazi, East Azarbayjan, West Azarbayjan, Kordestan Khorasan and Golestan provinces during 2004 to 2007. Soil and root samples were processed by the Whitehead tray method to extract of root lesion nematodes and Fenwick can method for cyst nematodes (Fenwick 1949, Whitehead and Hemming 1965). The extracted nematodes were identified based on morphological and morphometric characters and the population densities determined for a given volume of soil and weight of roots. Fungi from plant samples were cultured on potato dextrose agar (PDA) and incubated in the dark for 5-7 days at 22-23C. Fungal colonies were then sub-cultured onto new PDA petri dishes for subsequent identification using morphological traits. Forty five soil samples from different provinces were analysed by standard soil analytical techniques for soil type, pH, organic matter and micronutrient concentrations (Lindsay and Norvell 1978, Walkley and Black 1934).

RESULTS

The CCN species, *Heterodera filipjevi*, *H. latipons* and *H. avenae,* were found in 34% of soil samples, ranging from 18 to 50% in different provinces. Population densities of CCN in some soil samples were more than 50 cysts 300/g soil. The root lesion nematodes, *P. thornei* and *P. neglectus,* were found in 13% and 40% of soil samples respectively; and *Pratylenchoides ritteri* (another species of root lesion nematode) was present in 6% of soil samples, mostly in the western provinces

Table 1. Prevalence and population densities of cereal cyst nematodes (*Heterodera*) and root lesion nematodes (*Pratylenchus* and *Pratylenchoides*) in soil and root samples collected in six provinces of Iran.

Province	***Heterodera* spp.**		***Pratylenchus neglectus***				***Pratylenchus thornei***				***Pratylenchoides ritteri***	
	Soil		Soil		Roots		Soil		Roots		Soil	
	%	cysts/300 g	%	no/250 g	%	no/5 g	%	no/250 ml	%	no/5 g	%	no/250 ml
West Azarbayjan	34	1-143 (17)[1]	44	10-440	28	10-1140	21	10-70	11	20-400	2	1500
East Azarbayjan	24	1-22 (23)	35	10-450	63	10-1200	13	10-330	12	10-1250	6	10-110
Markazi	18	3-60 (22)	35	10-250	36	10-4230	-	-	-	-	-	-
Hamadan	50	3-140 (32)	58	10-700	47	10-3400	8	40-300	8	10-80	3	20
Kermanshah	32	2-63 (22)	36	10-360	30	10-1200	14	20-80	6	40-110	20	20-310
Kordestan	47	1-76 (18)	35	10-1640	36	10-1860	11	20-170	13	30-300	6	10-70

[1]*Heterodera* spp. numbers are full cysts with mean in parentheses

(Table 1). Root rot pathogens found in 45 plant samples assessed included *Fusarium pseudograminearum*, *F. culmorum*, *Bipolaris sorkokinana* and *Rhizoctonia cerealis* (being the causal agents of crown and root rots found in 35% of crown and root tissues) and frequently in combination with RLN and/or CCN. *Fusarium* species were the most isolated genera of fungi, being found in over 13% of root and crown tissue. In 87% of soil samples, soil organic matter concentration was less than 1%, the recommended level for healthy soil (Table 2). Soil pH was neutral to slightly alkaline (7.4 to 8.0) and most soils were clay loam or sandy loam. The micronutrients Cu and Mn were not found at concentrations considered deficient, however 11 and 20% soil samples were deficient in Fe and Zn (Table 2).

Table 2. Number (n=45) and proportion (%) of plant samples with fungal root rot pathogens and soil samples with low Fe, Zn and organic matter in a survey covering six provinces of Iran.

	Samples	**Proportion**
Fusarium culmorum	6	13.3
Fusarium pseudograminearum	5	11.1
Bipolaris sorokinana	2	4.4
Rhizoctonia cerealis	1	2.2
Iron deficient soil (<4.5 ppm Fe)	5	11
Zinc deficient soil (<0.5 ppm Zn)	9	20
Low organic carbon (<1%)	39	87

DISCUSSION

Overall, *H. filipjevi* was the most frequently and widely distributed species of the three CCN species found. However, *H. latipons* was the most prevalent species in two provinces. *H. avenae* was only detected in Kermanshah province and had high frequency in that region. Among the RLN species, *P. neglectus* was more prevalent in soil and root samples compared to *P. thornei*. The population densities of CCN and RLN exceeded the critical threshold for damage in a proportion of samples, so some crop losses could be expected. However, damage caused by nematodes and consequent yield reduction is known to be related to a range of factors in addition to population density, such factors as crop cultivar, soil temperature, moisture and texture, nematode pathotype and ecotype, and regional climatic conditions (Rivoal and Cook 1993, Smiley 2005).

High frequency of isolation of crown and root rot pathogens from plant samples is an indication of the presence of these causal agents in cereal fields in Iran. Therefore, under stress conditions usually occurring in rain-fed production, crop loss could be expected.

The beneficial effects of soil organic matter are well known, it has been demonstrated that organic matter positively affects soil water-holding capacity, and also that a loss of soil organic matter of 1 t/ha can cause reduction of wheat yields of 15 to 40 kg/ha (Bauer and Black 1994). In our study, 87% of soil samples had organic matter content of <1%, the minimal acceptable level. Fe and Zn are

important in root structure and strength, and there is a possibility that both of these micronutrients would interact with soil borne pathogens.

In conclusion, soil borne pathogens are widespread in many wheat growing areas in Iran and could potentially be causing yield loss under rain-fed cereal production. Research to further determine the economic importance and to develop control of these root pathogens are underway.

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PRESENT STATUS OF THE CEREAL CYST NEMATODE (*HETERODERA AVENAE*) IN SAUDI ARABIA*

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SUMMARY

The cereal cyst nematode (CCN), *Heterodera avenae*, was first reported from an irrigated wheat field in Saudi Arabia in 1987. *H. avenae* has since spread quickly to become a major limiting factor in wheat production. The nematode is now recognised as a damaging pathogen of wheat and barley, especially in Al-Kharj, Hail and Al-Qassim, the three major wheat-producing regions in Saudi Arabia. Several studies have been conducted on this important pest in our wheat fields. The Saudi populations of CCN were identified as *H. avenae*, based on morphological and morphometric features. The protein pattern of CCN from Al-Kharj and Rihyad regions matched that of typical *H. avenae*. The pathotype of the nematode was identified as Ha21. However, the PCR-RFLP and sequences of ITS-rDNA of six populations of *H. avenae* collected from Al-Qassim and Riyadh regions, distinguished three groups. Growth and physiology of infected wheat and barley were found to be adversely affected, and yield losses reached 92% in some heavily-infested sites in the Riyadh region. Farmers have been advised to follow crop rotation as a suitable control method. Forty genetically-diverse wheat genotypes have been screened for resistance to a local population of *H. avenae*. This material was also screened for resistance genes, using gene specific primers. Preliminary results indicated that all are susceptible to the local pathotype. However, the Saudi genotypes tested were generally less susceptible and had relatively higher yields compared to the check cultivars Irena and Yecora Rojo. All genotypes varied in their content of *Cre* genes. *Cre3* gene was found in some genotypes that showed resistance in the field. This gene could provide suitable resistance to the CCN pathotype in Saudi Arabia, and will be used in a marker-assisted selection to identify CCN-resistant wheat lines potentially suitable for use in Saudi Arabia.

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*Al-Hazmi AS, Dawabah AAM (2009) Present status of the cereal cyst nematode (*Heterodera avenae*) in Saudi Arabia. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 56-60. (CIMMYT: Ankara, Turkey)

INTRODUCTION

The cereal cyst nematode (CCN), *Heterodera avenae*, was reported on wheat and other cereals in many countries with different climatic types throughout the world (Sturhan and Rumpenhorst 1996). Yield losses in an intolerant wheat cultivars can range from 30 to 70% (Fisher 1982). However, damage is often more severe in rain-fed crops in soils with poor nutrient status, as in Saudi Arabia, where yield of wheat was reduced by as much as 40 to 92% and barley by 17 to 77% (Ibrahim *et al*. 1999).

HISTORY

Heterodera avenae was first reported from an irrigated wheat field in Saudi Arabia in 1987 (Youssif 1987). *H. avenae* has spread quickly to become a major limiting factor in wheat production in Saudi Arabia (Al-Hazmi *et al*. 1999). Year after year, this nematode has become recognised as a damaging pathogen of wheat and barley, especially in Al-Kharj, Hail and Al-Qassim, which are the three major wheat-producing regions of Saudi Arabia (Al-Hazmi *et al*. 1994, El-Meleigi and Al-Rokaibah 1996). Many of the *H. avenae*-infested wheat fields in Saudi Arabia are rotated with potato. Surprisingly, we have found that the nematode has also been spreading, in addition to the other known means, with the soil particles adhering to the potato seed tubers produced in such infested fields (Dawabah and Al-Hazmi 2007).

The origin of the *H. avenae* populations in Saudi Arabia is not known. The *H. avenae* populations from the three wheat-producing regions appear to have the same novel virulence phenotype (Al-Hazmi *et al*. 2001), suggesting that these populations might be indigenous or introduced from a single source.

STATUS

Saudi populations of CCN were identified as *H. avenae*, based on morphological and morphometric features by Al-Hazmi *et al*. (1994). The protein pattern of a Saudi population of *H. avenae* from Al-Kharj region matched closely that typical of *H. avenae* (Sturhan and Rumpenhorst 1996). The pathotype of the nematode was then identified as Ha21 (Al-Hazmi *et al*. 2001, Cook and Al-Hazmi 1997, Al-Hazmi and Ibrahim 2000). The PCR-RFLP and sequences of ITS-rDNA of six populations of CCN collected from Al-Qassim and Riyadh regions were studied by Al-Rehiayani (2007). The smallest genetic similarity was found between the populations of Unizah and Riyadh. However, populations of Zolfy, Cherry and Buraydah were the most similar. The dendrogram constructed using UPGMA analysis, distinguished three groups of populations in Al-Qassim and Riyadh regions (Al-Rehiayani 2007).

There is always a need for screening for resistance. Several wheat and barley genotypes were screened for resistance to some local populations of *H. avenae* (Al-Hazmi *et al*. 1994). Unfortunately, all tested cultivars of wheat (cvs West bread, Yecora Rojo, E 1-93-4 and L9) and barley (cvs Beacher, CC 89, Justo and Lignee 640) were found to be susceptible to the local CCN pathotype. The nematode has also been detected attacking roots of the Italian rye grass, *Lolium multiflorum* (known as multimo grass in Saudi Arabia and cultivated as a fodder crop) and wild

or foxtail barley, *Hordeum murinum* (a tufted annual grass that appears widely in winter) (Dawabah *et al*. 2007). Under Saudi field conditions, the life cycle of the nematode takes about 74 d on wheat and 64 d on barley (Al-Hazmi *et al*. 1997).

Forty wheat genotypes (some of them were developed by King Saud University) were screened for resistance to a local population of *H. avenae* in an outdoor pot experiment. All the tested genotypes were found to be susceptible (no. females/plant > 3) to the tested population (Al-Hazmi *et al*. unpublished data). However, Saudi wheat genotypes were generally less susceptible to the local *H. avenae* population, and had relatively higher yields, compared to the check cultivars; Irena and Yecora Rojo (Dawabah *et al*. unpublished data). The 40 genotypes tested were also screened for resistance genes (Al-Doss *et al*. unpublished data), using specific primers developed for known resistance genes. Microsatellite markers linked to *Cre1*, *Cre3*, *Cre5*, *Cre8*, *CreX*, and *CreY* genes were used to identify these genes. Results indicated that the tested wheat genotypes varied in their content of *Cre* genes. *Cre3* gene was found in few genotypes that showed resistance in the field. This gene could provide resistance to CCN present in Saudi Arabia, and will be used in a marker-assisted selection to identify CCN-resistant wheat lines potentially suitable for use in Saudi Arabia.

IMPACT

Growth and physiology of infected wheat and barley crops were found to be adversely affected (Al-Yahya *et al*. 1998), and yield losses was found to be as much as 92% in some heavily-infested sites of certain wheat fields in Riyadh region, Saudi Arabia (Ibrahim *et al*. 1999). As the nematode spreads all over the country, yield losses also increased, and the problem became national. Consequently, Saudi Agricultural Bank, in 2003, decided to cooperate with the wheat growers in scheduling their debts. In 2003, The Minister of Agriculture formed a specific committee to study and solve the problem. Therefore, a breeding program was recently initiated in the college of Food and Agriculture Sciences to select resistant lines to this pest.

PROSPECTS

In a current research project sponsored by the Center of Excellence in Biotechnology Research, King Saud University, we are planning to achieve the following research points: 1) morphological and molecular characterisation of CCN populations collected from the wheat-producing regions in Saudi Arabia, 2) pathotype characterisation of the Saudi CCN populations, 3) identification and mapping quantitative trait loci (QTLs) for CCN resistance in F2 population, and 4) selection of the most highly productive wheat lines with significant resistance to the cereal cyst nematode under Saudi field conditions.

RECOMMENDATION

Crop rotation, host resistance (O'Brien and Fisher 1974) and host tolerance (Fisher *et al*. 1981) have proven to be the only economically and environmentally sustainable methods for controlling damage caused by *H. avenae* to cereals. In Saudi

Arabia, we recommend crop rotation as the most suitable and best available control method. However, wheat-growing agricultural companies are reluctant to follow crop rotation as a suitable control method for commercial reasons. More studies are urgently needed on the biology, spread and management of this damaging pest in our wheat fields.

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OCCURRENCE AND DISTRIBUTION OF CYST NEMATODES INFECTING CEREALS IN SICILY, ITALY*

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SUMMARY

During 2008 and 2009, a survey on specific composition, frequency and geographical distribution of cyst nematodes living on cereals was conducted in Sicily, Italy. *Heterodera latipons* and *Heterodera hordecalis* appeared to be the most common species in durum wheat (*Triticum durum*) and barley (*Hordeum vulgare*) samples. Less widespread was *Heterodera avenae*, which occurred in a few fields of durum wheat. Laboratory investigations on soil samples and roots revealed the presence of all developmental stages for each species detected, with a marked preponderance of adult females and cysts. Symptoms and damage accompanying infestations by the above cyst nematode species were homogeneous in all fields and crops investigated: infected plants were stunted, small and scrubby and possessed chlorotic leaves and small roots.

INTRODUCTION

Cyst nematodes attack many species of cereals, frequently causing serious yield losses in major food crops. In particular, nematodes in the *avenae* group of the genus *Heterodera* occur in all main areas of cereal production in the Mediterranean Basin and cause substantial economic losses. They are major pests of cereals throughout the world as well (Handoo 2002). The taxonomy and diagnostic morphological characteristics of the group have been developed by numerous scholars: cysts are lemon-shaped and their colour is dark-brown to black and vulval cones are characterised by fenestration.

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Following infections by nematodes of the *avenae* group, plants are stunted, small and scrubby and possessed small roots and chlorotic leaves. In view of the economic significance of cereal crops (mainly wheat, oats, barley and forage grasses) in Sicily, a growing importance must be accorded to cyst nematodes in this area. In this light, the main target of this study was to investigate the occurrence and distribution of cyst nematodes infecting cereals in Sicily.

METHODS

The survey was carried out over two years (2008-2009) in the main cereal growing areas of Sicily. Samples were taken especially where plants showed chlorotic and yellowing leaves, poor growth and reduced production, and therefore were suspected to be attacked by nematodes of this group. Each sampled area was 1 ha in size, and each sample was a composite of 30 subsamples collected with a small spade in the plant rhizosphere, and at a depth of 5-20 cm after removing the top 5 cm soil. The entire sample was thoroughly mixed and 2 kg of sample kept in a plastic bag and taken to the laboratory. Samples were stored at 6C until they were processed. Both durum wheat (*Triticum durum*) and barley (*Hordeum vulgare*) crops were samples by the same method. Roots were carefully washed free of adhering soil and a portion was observed under a stereomicroscope (25x magnification) to ascertain the presence of nematodes; females were subsequently dissected. Soil samples were then thoroughly mixed, air dried and processed with a Fenwick can to extract cysts. Emerging second-stage juveniles were killed by gentle heat, fixed in triethanolamine-formalin (TAF) solution and mounted in anhydrous glycerol on permanent slides. Cone mounts of cysts, previously cleaned, were also prepared and mounted in Canadian balsam.

The identification process was based on cyst morphology, namely on posterior regions (cone mounts), and on morphology of 30 second-stage juveniles, namely body length, the length of the region around the tail and the shape and length of the stylet. These morphometric measurements were compared with those of other populations of various species of the *avenae* group and other cyst forming nematodes collected on Poaceae deposited in the USDA Nematode Collection at Beltsville, Maryland.

RESULTS

Specimens of the *avenae* group were abundant in both soil and root samples of the aforementioned crop plants. Morphological examination of cyst vulval cones and second-stage juveniles revealed the presence of three species: *H. latipons*, *H. hordecalis* and *Heterodera avenae.*

Adult females of *H. latipons*, discovered for the first time in Italy (Veneto) in 1975 (Tacconi 1976), are lemon-shaped; some individuals are smaller or larger compared to the typical shape. Females show a distinct neck and a prominent vulval cone. The cuticle is characterised by a thin sub-crystalline layer; females are pearl white in colour and later turn gradually to dark brown cysts. Vulval cones (Figure 1c) are characterised by a short vulval slit (6-9 µm), two different translucent areas called fenestrae, a strong underbridge with a pronounced thickening in the middle and a bifurcation at both ends and sometimes a few bullae, as reported by Tacconi (1976)

on other Italian populations of *H. latipons*. The second-stage juveniles are vermiform with a rounded and conical tail (tail length 48-57 μm). The stylet is well developed (stylet length 23-25 μm) with basal rounded knobs which display a typical anchor shape (Figure 1a). All other morphological details agree with the typical characters of *H. latipons* (Franklin 1969) as described by Handoo (2002).

H. hordecalis (Figure 2) morphological characteristics (Andersson 1974) are quite similar to those of *H. latipons.* Nevertheless, they display fine distinctive features: the most important distinguishing character between *H. latipons* and *H. hordecalis* is

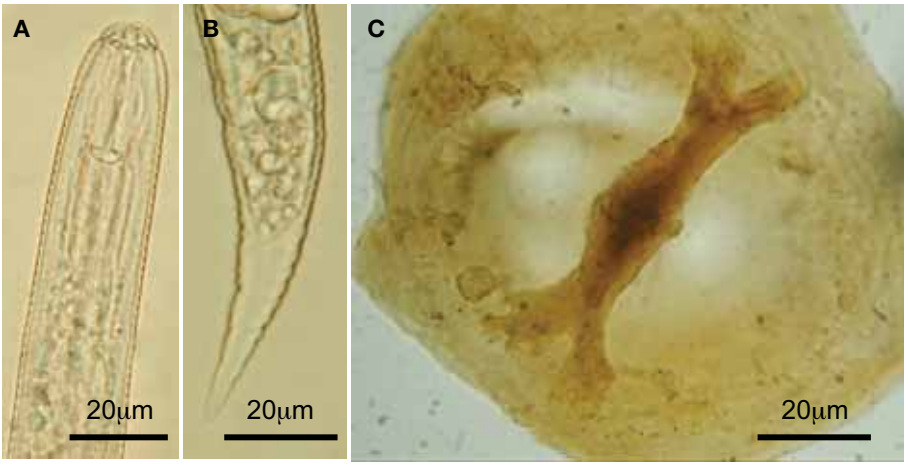

Figure 1. Photomicrograph of *Heterodera latipons*. A, head and B, tail of second-stage juveniles, C, cyst vulval cone pattern with underbridge and bullae.

the vulval slit (Figure 2c), which in *H. hordecalis* is bigger than in *H. latipons.* In *H. hordecalis*, the vulval slit is about 17-25 μm; moreover *H. hordecalis* differs from the above described *H. latipons* because its underbridge is less sclerotised than in *H. latipons*. The mentioned distinctive morphological details agree with the typical characters of *H. hordecalis* (Andersson 1974).

H. avenae cysts were dark brown to black, ambifenestrate, bullae and underbridge prominent with a shorter vulval slit 9-10 μm long; the second-stage juveniles had a body length of 530-553 μm, stylet measured 24-26 μm, stylet knobs were shallowly concave anteriorly, tail measured 50-56 μm and hyaline tail terminus was 30-36 μm long. All these and other morphological and morphometric details for *H. avenae* were consistent with those given by Handoo (2002).

DISCUSSION

Because symptoms and damage caused by the above-mentioned nematodes are not specific only to nematodes, nematode diagnosis based on symptomatology is difficult. The stunting and yellowing may have often misled farmers to erroneously attribute the symptoms to other causes, such as drought, iron or other nutrient deficiencies, chlorosis or other plant pathogens. This may be why the species in this study have remained undetected for several years in Sicily.

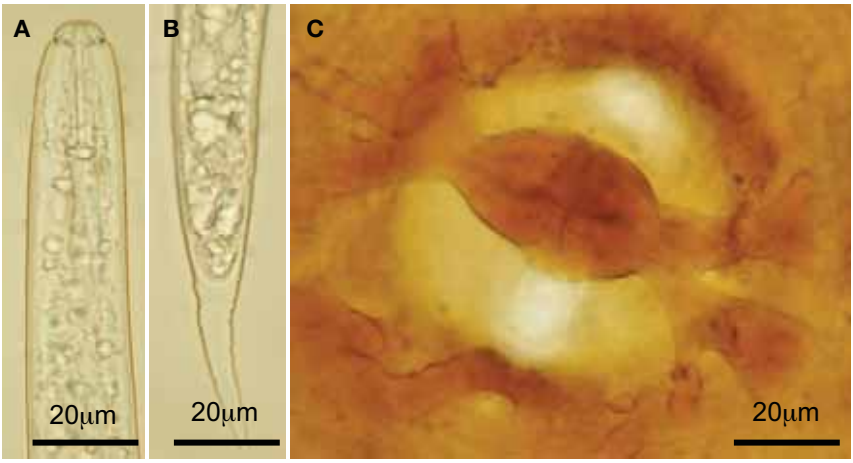

Figure 2. Photomicrograph of *Heterodera hordecalis*. A, B, head and tail of second-stage juveniles; C, cyst vulval cone pattern with underbridge and bullae.

Heterodera spp. do not cause definite symptoms on cereals when present at low population densities in the soil. Only when *Heterodera* populations increase above a crop's tolerance limit (Potter and Olthof 1993) do symptoms of nematode infection become sufficiently obvious to concern farmers and plant pathologists about the consequent damage and economic losses *Heterodera avenae* and *H. latipons* produce only one generation per host plant growing season; within 4 months at 6C but only 40 d at 18C (Mor *et al.* 1992). Nematodes of the *avenae* group have been studied in Italy (Inserra *et al.* 1978, Greco and Brandonisio 1987) to provide biological information and to suggest control strategies. As to *H. avenae* on wheat, Greco and Brandonisio (1987) concluded that soil containing over 35% clay and other environmental conditions in southern Italy are responsible for maintaining the nematode populations at very low levels and therefore for the apparent absence of damage. We observed that major crop damage was strictly related to the peculiar environmental conditions of Sicily and to soil with very low clay content and high sand content. This combination of environmental conditions and heterogeneity in soil composition over different areas accounts for the variation in nematode-inflicted crop damage between different fields of cereals.

ACKNOWLEDGMENTS

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CYST NEMATODES OF THE GENUS *HETERODERA* IN BELGIUM*

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SUMMARY

Information about the presence of *Heterodera* species in cultivated fields in Belgian conditions is scarce and scattered. Only the presence of *H. schachtii* has been well documented as this nematode is causing considerable yield reductions in the sugar beet industry. Several *Heterodera* species were reported in the updated list of Belgian nematodes (2003): *H. avenae*, *H. goettingiana*, *H. cruciferae, H. humuli, H. trifolii*. Two surveys, one in arable fields where cereals are grown (2007) and another on turf grass in golf greens and football fields (2006) revealed the presence of *H. mani* (both surveys) and *H. ustinovi*, *H. bifenestra* and *H. hordecalis* (turf grass). The clover cyst nematode *H. trifolii* was also found in the arable field survey and the carrot cyst nematode *H. carotae* was suspected in one field, but its identity needs to be confirmed. Also *H. betae* has been reported occasionally. This nematode can be easily confused with *H. schachtii,* the most dominant *Heterodera* species (60% of Flemish fields infested). The presence (or absence) of cereal cysts should also be investigated in the southern part of the country (Wallonia) and the reasons for its scarcity should be investigated, especially in view of climate change. The high incidence of *H. schachtii* – in remarkable contrast with the low prevalence of cereal cysts – needs more attention in terms of management. Investigation of resistance mechanisms and breeding of resistant cultivars of beets and other host crops (e.g. rapeseed) is needed, together with the study of the influence of possible pathotypes and environmental conditions on population development. In addition, there is an overall need for more rapid, though accurate, molecular identification tools, so that large surveys can produce a maximum of information in a short time frame.

*Viaene N, Çolak Yılmaz Z, Deeren AM, de Sutter N, Vandenbossche B, Bert W (2009) Cyst nematodes of the genus *Heterodera* in Belgium. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 66-70. (CIMMYT: Ankara, Turkey)

INTRODUCTION

Cyst nematodes (mostly *Heterodera* spp. and *Globodera* spp.) are restricting yields in many crops all over the world. Their ability to withstand desiccation in the protective cyst stage enhances their dispersal and survival. The *Heterodera* genus contains over 60 species which can multiply on a great range of host plants. Some host plants are economically important crops in Belgium, such as potato, cereals and sugar beet.

Cereal cyst nematodes (CCN) are causing yield losses in many parts of the world (Europe, USA, Australia and developing countries). Important CCN species are *Heterodera avenae, H. filipjevi, H. latipons* and *H. hordecalis.* In Belgium, only *H. avenae* has been reported from cultivated soils and meadows (Bert *et al*. 2003). The most widely grown cereal in Belgium is wheat: it is grown on about 60% of the 325,000 ha used for cereal production (Anon. 2007)**.** Wheat yields are very high (around 8 t/ha) and yield reductions due to plant pathogens are mainly caused by fungi; nematodes are not reported as a problem. In other northern European countries, however, damage in grain crops due to CCN has been reported. Mainly *H. avenae*, but lately also *H. filipjevi*, were found to cause reduced growth in cereals (Holgado *et al*. 2006, Mitchinson *et al*. 2008).

The white beet cyst nematode, *H. schachtii*, is well known as a problem in the sugar beet industry in Belgium (Hermann and Wauters 2005). Although the cultivation of sugar beets has decreased in recent years, there is an increased interest in breeding for resistant and tolerant cultivars. This is mainly encouraged by the ban on the nematicide aldicarb since 2008.

To know more about the occurrence of *Heterodera* spp., in particular cereal cysts, a survey in cereal fields was organised in 2007. This survey, as well as another survey on plant-parasitic nematodes in turf grass in 2006 (Vandenbossche *et al*. 2008), revealed the presence of several *Heterodera* spp.

HISTORY

Research on *Heterodera* spp. in Belgium has mainly focused on the white beet cyst nematode *H. schachtii*. This species reduces beet yields considerably (up to 50%) and is present in about 60% of the fields (Hermann and Wauters 2005, Çolak-Yilmaz *et al*. 2009). Investigations on this nematode consisted of the evaluation of nematicides, studies of the damage threshold and of the population dynamics in rotations with susceptible, resistant and tolerant cultivars and green manures (Hermann and Wauters 2005, G. Legrand personal communication).

No research has been conducted on cereal cysts as these are not considered as a major problem. Only general reports of surveys on nematodes (Bert *et al*. 2003, Vandenbossche *et al*. 2008) and information obtained through diagnostic labs shed some light on the occurrence of these and other *Heterodera* species. Most research on *Heterodera* in Belgium has focused on molecular identification methods (Perry *et al.* 2007).

STATUS

Cereal cyst nematodes

Heterodera avenae was recorded in Belgium from cultivated soils and meadows (Bert *et al.* 2003). This nematode was first recorded in 1989, but its finding probably dates back to earlier days.

In 2007, a survey was organised to retrieve additional information about the occurrence of this and other CCN (Çolak-Yılmaz *et al*. 2009). This survey was limited to the northern part of Belgium (Flanders), where about 145,000 ha of cereals are cultivated. Soil samples were taken at 22 locations in 50 fields where cereals (winter wheat, winter barley and maize) are grown in rotation with beets, potato and vegetables (carrot, pea, bean, parsley and turnip) or rapeseed. Only cysts and no mobile nematodes stages were extracted from a 1.5-kg subsample of the 112 soil samples retrieved from the fields. No typical cereal cysts were found in this survey. Also, in the Diagnostic Centre for Plants (DCP) at ILVO, cereal cysts have never been detected as the cause of bad plant growth in samples submitted by farmers for diagnosis. *Heterodera mani* was found in 3 fields of the survey, and is sometimes found in field samples in the DCP, but no damage on cereals has ever been associated with this nematode. The host plants of *H. mani* are restricted to grass species (Mathews 1971).

In a survey for plant-parasitic nematodes in golf greens and football pitches, where 42 samples were taken at 16 locations in Flanders, several species of *Heterodera* where found; *H. mani*, *H. ustinovi*, *H. bifenestra* and *H. hordecalis* (Vandenbossche *et al*. 2008). In this survey, only mobile stages were extracted from the soil.

Other cyst nematodes

The most dominant species in the survey in arable fields in 2007 was the white beet cyst nematode, *H. schachtii*; it occurred in 30 (60%) of the fields sampled. *Heterodera betae*, the yellow beet cyst nematode, was not detected in this survey. However, this cyst nematode, which is able to multiply on cultivars of beet (both resistant and tolerant to *H. schachtii*), yellow mustard and rapeseed, has been found occasionally in Belgium (Amiri *et al*. 2003, DCP-ILVO unpublished data). Possibly this species is more widespread than is known because it is difficult to differentiate from the white beet cyst nematode, especially when its identity is assumed to be *H. schachtii* when found on beet.

Other *Heterodera* species present in Belgium are *H. trifolii* (found in 12% of the 50 fields in the survey), *H. goettingiana*, *H. cruciferae* and *H. humuli* (Bert *et al*. 2003, Çolak-Yilmaz *et al*. 2009). Their most well-known host plants are clovers, pea, cabbage and hops, in the same order of mentioning. During the 2007 survey, cysts suspected to be *Heterodera carotae* were found in one field in which carrots were part of the rotation, but the identification needs to be confirmed.

IMPACTS

As CCN were not found and no yield losses are reported, we can conclude that these nematodes have currently no impact on cereal production, at least not in Flanders. In contrast, there is a great impact of the beet cyst nematode, *H. schachtii* on beet

production. This nematode is widespread, reaches high infestation densities and causes yield reductions. The impact of the other *Heterodera* spp. found in the surveys is most probably of no economic importance, yield loss might occur only in some cases when heavy infestations are present in the field. So far, crop damage has only been reported for *H. betae* in pea (DCP-ILVO unpublished data).

PROSPECTS

Cereal cyst nematodes apparently pose no problem to Belgian cereal crop production. We have to watch out, however, that we are not overlooking the problem because it has never occurred before. The increased damage in cereals due to *H. avenae* and *H. filipjevi* in Norway and in the UK should make us aware that a similar scenario might be possible in Belgium. New pathotypes of the cyst nematodes, the use of new wheat cultivars or a change in climatic conditions could provoke such events.

It is obvious that many aspects of the beet cyst nematodes still need to be investigated. It is assumed that *H. schachtii* will continue to be a problem as long as no new efficient nematicides are available and resistant cultivars are not widely used. Moreover, *H. betae* might become a problem when no careful identification is performed and host plants resistant to *H. schachtii*, but not to *H. betae*, are grown.

RECOMMENDATIONS

It is recommended to expand the survey to the Walloon part of Belgium, where cereals are grown on greater area (180,000 ha) than in Flanders (145,000 ha) (Anon. 2007). When doing so, more cysts per sample should be identified. In the survey in arable fields (Çolak-Yılmaz *et al*. 2009) only 10 cysts per sample were used as it is difficult and time-consuming to identify *Heterodera* spp. up to species level based on morphological and morphometric features. Therefore, it is also strongly recommended to further develop molecular identification tools so that fast and accurate species determination can be performed on as many individuals as possible. If indeed no or very few cereal cyst nematodes are present all over Belgium, it would be very interesting to find out why cereal cyst nematodes do not thrive, but beet cyst nematodes are almost ubiquitous. Cereal production in Belgium is successful because of the favourable climatic conditions with enough long periods of rain and also because of the good quality of the soils. Perhaps these soils harbour antagonistic fungi or other organisms that suppress the development of cereal cyst nematodes. Climatic change might influence these conditions and result in an increase of CCN. Possibly the absence of cereal cysts could simply be due to the cultural practices (rotation and choice of cultivars) applied in Belgium.

The beet cyst nematodes pose the greatest problem in Belgium. Research on beet cyst nematodes should be aimed at the development of rapid and accurate identification methods, at studies of the resistance mechanism in tolerant and resistant cultivars of beet and other host crops, and at the breeding of these new cultivars. The possible existence of pathotypes with different reaction to the tolerant and resistant cultivars should be kept in mind when performing this research, as well as the influence of climatic conditions on resistance and on nematode population development.

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PART 2 LOCAL STATUS

STUDIES ON MORPHOMETRIC VARIATION WITHIN THE POPULATION OF *HETERODERA ZEAE* ON CEREALS IN INDIA*

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SUMMARY

An intensive survey was carried out during autumn 2004 and spring 2004-05 in different agroclimatic regions of Rajasthan, India. In total, 1087 plant root and soil samples were collected from different localities and hosts (wheat, maize and barley) to study morphometric variation within population of *Heterodera zeae* associated with these crops. Wheat plants grown in maize cultivated fields has been recorded as a new host in the state of Rajasthan. Body dimensions of second stage juveniles, cyst length, cyst width and vulval cone top characters were measured. Comparisons of body dimensions of the populations recovered with the original species description showed that most of the dimensions of vulval cone top characters were within the diagnostic range. However, in some populations, characters like width of vulval bridge, underbridge length and width showed variations, which was recorded 37.8% higher than original value in width of vulval bridge toward higher side, variations in under bridge length was 3.9% toward lower side, whereas, in under bridge width it was 7.6% towards higher side, cyst length of few population of *H. zeae* showed variation up to 13.5% toward lower side. For second stage juveniles, characters like body length and stylet length were more variable. The variation in body length was 2.2% towards higher side and in style length up to 1.7% towards higher side. The variations in body dimensions of second stage larvae and vulval cone top characters within the population of *Heterodera* spp. collected from different agroclimatic regions of the state may be due to varied soil type, prevailing temperature, soil moisture, preceding crops and several other abiotic and biotic factors.

*Sharma SK, Siddiqui AU, Parihar A (2009) Studies on morphometric variation within the population of *Heterodera zeae* on cereals in India. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 73-78. (CIMMYT: Ankara, Turkey)

INTRODUCTION

Cyst nematodes represent a large group of plant parasitic nematodes comprising more than 100 species. These species are presently included in six genera of the family Heteroderidae; *Heterodera*, *Globodera*, *Cactodera*, *Punctodera*, *Dolichodera* and *Afenestrata.* In nematology, morphology has been the basis for identification and systematics. In cyst nematodes, several life stages are important and useful in both morphology and identification. The cyst and its enclosed juveniles and eggs are of greatest value and are the most widely used. In the present study the morphological and morphometric characters were taken into consideration.

METHODS

A systematic survey was conducted from 2004 to 2006 during both autumn and spring. In total, 1087 plant root and soil samples were collected from different localities and hosts (wheat, maize and barley) to study morphometric variation within population of *Heterodera zeae* associated with these crops. Measurement of cyst length, cyst width, vulval cone top structures like fenestral length, fenestral width, semi fenestral length, vulval slit length, width of vulval bridge, underbridge length and under bridge width (Franklin 1969) and body dimensions of second stage juvenile like body length, body width, stylet length, tail length, hyaline tail terminus length, ratio of tail length and hyaline tail terminus length, and ratio of tail length and body with anus (De Man 1880) were taken into consideration to compare it with the original description of this species.

RESULTS

Twenty four populations of *H. zeae* were recovered from different agroclimatic zones of Rajasthan (Table 1). Results showed that cyst length, cyst width, vulval cone top structure and body dimensions of second stage juveniles (J2) exhibited much variability within the different populations of *H. zeae*. Comparison with original description of species (Golden and Mulvey 1983), the characters like width of vulval bridge, fenestral length, fenestral width, vulval slit length, underbridge length and underbridge width showed variation toward the lower and higher sides of the described range.

Variation in cysts and vulval region is shown in Table 2. Variation in width of vulval bridge was up to 61% in CH-6 followed by 37.8% in RAJ-51 and 30.9% in UDR-69 towards highest side. Variations in fenestral length was 4.6% in UDR-121 towards higher side, while 3.4% in UDR-241 towards lower side.

Variation in fenestral width was 16.6% in CH-6, 8.5% in UDR-69 towards the lower side. Variation in vulval slit length was 24.5% in CH-6, 22.5% in CH-65 and 17.3% in UDR-2 towards lower side, while it was 18.1% in UDR-161. Similarity variation in underbridge length was 11.4% in Bansw-7 and 9.9% in UDR-45 towards higher side of the original value. Variation in underbridge width was up to 86.6% in UDR-75, 82.1% in UDR-45 and 60.3% in UDR-6 towards higher side.

Variation in cyst length was 37.2% in Raj-51 32.4% in 241 and 26.1% in CH-74 towards the lower side when compared to the described range. Variations in cyst

Table 1. Populations of *Heterodera zeae*, corn cyst nematode recovered from different localities of Rajasthan, India.

Code	Locality	Code	Locality
BANSW-7	Borwat Farm, Banswara	UDR-45	Kavita, Udaipur
BKR-1	Vallabhgarden, Bikaner	UDR-52	Badgavn, Udaipur
CH-6	Kapasan, Chittorgarh	UDR-69	Gudli, Udaipur
CH-65	Bhadsoda, Chittorgarh	UDR-75	Sesarma, Udaipur
CH-74	Menar, Chittorgarh	UDR-86	Bhatewar, Udaipur
CH-98	Delwas, Chittorgarh	UDR-93	Dabok, Udaipur
CH-114	Railmagra, Chittorgarh	UDR-106	Takri, Udaipur
RAJ-26	Nathdwara, Rajsamand	UDR-113	Thoor, Udaipur
RAJ-51	Kankroli, Rajsamand	UDR-121	Sesarma, Udaipur
UDR-2RCA	Nem Farm Udaipur	UDR-161	Kadia, Udaipur
UDR-6	RCA, Breeding Farm, Udaipur	UDR-193	RCA Farm, Udaipur
UDR-29	Chikalvas, Udaipur	UDR-241	Salumbar, Udaipur

width was 17.1% in Raj-51, 9.9% in CH-98 and 4.5% in UDR-193 towards the lower side of the original range.

Variation in juvenile morphology is shown in Table 3. Variation in J2 body length was 13.2% in CH-6, 8.0% in CH-114 and 5.9% in UDR-45 towards the lower side. Variation in J2 body width was up to 22.4% in CH-6 followed by 17.9% in UDR-45 and 17.5% in UDR-2 towards its lower side. While this variation was 36.7% in UDR-113, 35.3% in UDR-93 and 33.0% in UDR-106 towards higher side of the described range.

Variation within stylet length were 31.8% in UDR-6 followed by 31.5% in UDR-29 and 30.8% in Raj-26 towards higher side of the described range.

J2 tail length showed variation of up to 15.3% in BKR-1 followed by 13.9% in UDR-193 and 11.5% in UDR-86 towards the lower side of its described range, and 29.4% variation in UDR-52, 11.8% in UDR-2 and 9.9% in UDR-6 towards the higher side were observed. Variations in J2 hyaline tail terminus length was up to 15.9% in UDR-6, followed by 11.9% in UDR-52 and 11.7% in UDR-69 towards higher side of the described range.

DISCUSSION

Morphometric variation occurring between populations of a nematodes species or between specimens within a population have been reported by various authors. The differences have been reported due to the geographical distribution, eco-phenotypic effects and different host (Goody 1952, Rohde and Jenkins 1957, Bird and Mai 1965, de Grisse and Loof 1970, Tarte and Mai 1976, Azmi and Jairaypuri 1978, Evans and Franco 1977, Tarjan and Frederick 1978).

Table 2. Morphometric variations within populations of corn cyst nematode (*Heterodera zeae*) collected from Rajasthan (n=10).

Population	Cyst (µm)		Cone top (µm)					
	Cyst length	Cyst width	Fenestral length	Fenestral width	Vulval slit length	Width of vulval bridge	Under bridge length	Under bridge width
Original description	565 428-785	380 245-525	46 39 -57	27.4 19-38	40.40 38-45	-	38.7 34-51	9.1 8-10
BANSW-7	307±7.1 298-314	239±5.4 230-245	42±0.67 40-42	22±1.35 20-23	37±0.92 35-38	9±0.36 8-9	56±0.64 55-57	15±0.48 14-15
CH-6	389±13.5 372-404	374±9.8 359-383	41±1.12 39-42	19±1.89 16-21	31±1.80 29-33	15±1.09 13-16	47±2.47 43-49	13±0.46 13-14
CH-65	413±6.2 404-421	329±8.3 318-339	55±1.10 53-56	27±2.03 25-30	31±1.51 29-33	7±0.75 6-8	44±0.69 43-45	10±0.60 10-11
CH-74	327±9.1 316-340	253±4.2 248-259	40±0.61 40-41	28±1.39 26-30	40±1.21 39-42	10±0.31 10-11	51±0.76 50-52	15±0.64 15-16
CH-98	352±2.3 350-356	273±4.6 266-278	44±0.95 43-45	27±1.41 26-29	39±1.34 37-41	12±0.36 12-13	50±1.08 48-51	11±0.88 10-12
RAJ-51	278±5.5 269-283	212±5.6 203-219	42±0.58 41-43	22±1.01 20-23	39±0.92 38-40	13±0.29 13-14	48±0.75 47-49	13±0.58 12-14
UDR-2	491±2.4 488-496	384±4.1 378-388	50±1.23 48-51	22±1.87 20-25	34±1.96 31-37	11±0.69 10-12	53±1.88 50-55	17±0.56 16-18
UDR-6	490±7.7 480-501	384±6.3 375-392	55±1.20 53-56	25±1.33 23-26	33±1.13 31-34	10±0.81 9-11	49±1.38 48-51	15±0.87 14-16
UDR-45	496±1.5 494-508	389±5.9 380-396	48±1.45 46-53	23±1.02 19-24	46±0.93 45-49	13±0.89 12-15	54±1.17 53-56	17±1.34 15-19
UDR-69	459±7.3 450-470	375±5.8 368-382	42±1.57 40-44	22±2.60 17-24	39±1.87 37-41	12±0.78 11-13	45±1.26 44-47	11±1.54 9-13
UDR-75	500±6.4 492-509	392±6.0 385-402	50±2.07 48-53	21±1.48 19-23	47±0.65 46-48	14±0.48 13-14	55±0.72 54-56	17±1.14 16-19
UDR-121	388±3.81 383-393	287±1.6 285-289	45±0.54 44-45	22±0.86 22-24	42±1.47 40-43	7±0.56 6-7	44±0.85 43-46	14±0.56 13-15
UDR-161	446±5.9 437-453	339±6.9 329-346	48±1.36 47-50	25±1.01 24-26	52±1.24 50-53	9±0.49 8-10	50±1.03 49-51	14±0.81 13-15
UDR-193	340±11 326-355	236±1.9 234-239	43±0.56 42-44	21±0.83 20-22	33±0.67 32-34	9±0.44 8-9	42±0.64 41-43	16±0.58 15-16
UDR-241	300±7.9 289-308	243±4.8 236-249	38±0.19 38-38	23±0.72 22-24	35±0.70 34-36	7±0.67 6-8	37±0.62 37-38	14±0.57 13-14
SEm	2.35	1.97	0.35	0.41	0.37	0.18	0.32	0.23
CD 5%	6.52	5.49	0.98	1.13	1.03	0.51	0.88	0.65
CV	1.82	1.99	2.39	5.22	3.04	6.30	2.11	5.49

Morphometric variation between the populations of heteroderid nematodes from various localities may reflect natural variation among the populations due to the adaptation of population to survive in that locality, which results in biotypes due to genetic drift in the population.

Table 3. Morphometric variations within populations of corn cyst nematode (*Heterodera zeae*) collected from Rajasthan (n=10).

Populations	Second stage juveniles (µm)				
	Body length	Body width	Stylet length	Tail length	Hyaline tail terminus length
Original description	423 392-451	19.7 20-20	45.9 20-20	45.9 40-51	22.70 18-26
BKR-1	378±2.9 375-382	16±0.81 15-17	22±0.73 22-23	37±2.14 34-40	19±0.79 18-20
CH-06	348±5.5 340-354	16±0.57 15-17	25±0.89 24-26	39±0.77 38-40	19±0.64 19-20
CH-114	366±3.4 362-370	22±0.79 21-23	21±0.81 20-22	41±1.01 40-42	24±0.82 23-25
RAJ-26	429±3.1 425-433	20±0.65 19-20	25±0.77 24-26	56±1.42 53-57	28±0.90 27-29
UDR-2	386±3.6 380-390	17.1±0.73 16-18	25±0.34 24-25	55±1.55 53-57	26±0.32 25-26
UDR-6	433±3.8 428-438	21±0.24 21-21	26±0.54 25-26	55±0.73 54-56.0)	30±0.51 29-30
UDR-29	447±4.9 440-453	18±0.71 17-19	25±0.79 24-27	45±1.38 43-47	22±1.58 20-24
UDR-45	376±7.9 364-385	17±0.46 16-17	20±0.89 19-21	40±0.94 39-42	20±1.27 19-22
UDR-52	416±3.6 411-421	18±0.52 17-18	25±0.41 24-25	65±0.44 65-66	29±0.43 28-29
UDR-69	416±5.2 410-422	19.8±0.53 19-20	24±0.61 24-25	50±1.03 48-51	28±0.67 28-29
UDR-86	383±2.9 379-387	17±0.78 16-18	23±0.69 23-24	38±2.01 35-40.	19±0.63 19-20
UDR-93	444±4.1 438-450	26±0.67 26-27	24±0.41 24-25	503±2.31 48-54	27.3±0.48 27-28
UDR-106	441±5.6 433-447	26±0.51 25-27	24±0.41 24-25	51±1.83 49-53	26.2±0.36 26-27
UDR-113	432±3.0 428-437	26±0.93 25-27	24±0.65 23-25	41±1.23 39-43	24.9±0.49 24-25
UDR-193	375±5.7 367-381	22±0.52 21-22	20±0.52 20-21	37±2.23 34-40	20±0.62 19-21
SEm	1.34	0.24	0.20	0.45	0.26
CD 5%	3.73	0.67	0.57	1.25	0.73
CV	1.05	3.51	2.77	3.13	3.45

Results obtained from present investigation are also in accordance with the findings of Singh and Khan (1981), Hirschmann (1956), Basu and Bagri (1985) and Kaushal and Seshadri (1987), who also reported 8.3 to 15.1% variation of both sides of original values with respect to body dimension. These variations are bound to *Heterodera* species, that were recovered in this present investigation in Rajasthan state, which has the extreme agroclimatic conditions that may cause extreme

variation in their morphometric values of this species. Therefore for identification of cyst nematodes, the extent of variation within the species must always be considered in species determination.

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OCCURRENCE AND DISTRIBUTION OF TWO SPECIES OF CEREAL CYST NEMATODES *HETERODERA AVENAE* AND *H. FILIPJEVI* IN KHUZESTAN PROVINCE, IRAN*

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SUMMARY

The distribution and population density of cereal cyst nematodes (CCN) in Khuzestan province were surveyed. A total of 144 composite soil and root samples were taken during 2008-2009. The survey found 37 and 35% of wheat and barley fields, respectively, in Ahvaz, Andimeshk, Baghmalek, Behbahan, Dasht-e-Azadeghan, Dezful, Gotvand, Hendijan, Izeh, Masjed Soleiman, Omidiyeh, Ramhormoz, Ramshir, Shadegan, Shushtar and Susa regions were infested with the CCN species, *Heterodera avenae* and *H. filipjevi*. Population densities of CCN in soil from wheat fields ranged from 1 to 103 (mean 18) cysts/100 g soil and from 0 to 1400 (mean 437) eggs and second stage juveniles (J2)/100 g soil. Population densities in barley fields ranged from 200 to 600 (mean 300) eggs and J2/100 g soil.

INTRODUCTION

Khuzestan province is located in southwestern Iran, bordering on the Persian Gulf and covering an area of 64,000 km^2. The province has a warm climate with an average temperature 31C summer and 15C in winter and average annual precipitation of 266 mm. The area under wheat and barley cultivation is 0.7 Mha with total production of 1.6 Mt, making it the second most important cereal producing province, contributing 17% of annual production in Iran in 2005-2006 (Anon. 2006). The cereal cyst nematodes (CCN) are a major pest of cereals throughout the world. *H. avenae*, *H. filipjevi*, *H. hordecalis* and *H. latipons* have been reported from cereal fields and grasslands in Iran (Tanha Maafi *et al*. 2007). However, there is not enough information on the status of CCN in cereal fields in

*Ahmadi AR, Tanha Maafi Z (2009) Occurrence and distribution of two species of cereal cyst nematodes *Heterodera avenae* and *H. filipjevi* in Khuzestan province, Iran. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 79-81. (CIMMYT: Ankara, Turkey)

Khuzestan. The aim of this study was to determine the occurrence, distribution and population density of CCN in wheat and barley in southwestern Iran.

METHODS

A total of 144 composite soil and root samples were collected from 19 cereal growing districts of Khuzestan province during 2008-2009. The root tissues of 10 wheat and barley plants from each sample were examined under a dissecting microscope to observe mature female and disease symptoms. Soil samples were processed by the Fenwick can method (Fenwick 1940) to extract cysts. The number of cysts was counted in each sample, as well as the egg and second stage juvenile (J2). The species were identified by morphological and morphometric characters (Wouts and Balwin 1998).

RESULTS

Distribution and population density of CCN in Khuzestan province are presented in Table 1. Of the 144 soil and root samples, 50 samples were infested with *H. avenae* and/or *H. filipjevi*. CCN being widespread in important cereal growing districts of the province, including Ahvaz, Andimeshk, Baghmalek, Behbahan, Dasht-e-Azadeghan, Dezful, Gotvand, Hendijan, Izeh, Masjed Soleiman, Omidiyeh, Ramhormoz, Ramshir, Shadegan, Shushtar and Susa. The population densities of CCN in soil from wheat fields ranged from 1 to 103 (mean 18) cysts/100 g soil and from 0 to 3300 (mean 394) eggs and J2/100 g soil. The highest and lowest incidence was found in Masjed Soleiman and Izeh with 103 and 1 cysts/100 g soil respectively. Population densities of CCN in soil from barley fields ranged 11 to 71 (mean 36) cysts/100 g soil and from 0 to 734 (mean 419) eggs and J2/100 g soil. The highest and lowest incidence was observed in Lali and Ramhormoz districts with incidences of 100 and 33%, respectively. CCN were found in wheat cvs Chamran, Verinak, Yavarus and Atila, local barley cultivars and the weeds, *Lolium prenne*, *Hordeum spontaneum* and *Avena ludovicians*.

H. avenae and *H. filipjevi* were found in 50 and 37.5% samples, respectively, and as mixed populations in 12.5% of samples.

DISCUSSION

The presence of *H. avenae* in Khuzestan province. as well as in western provinces of Iran, had already been reported (Ahmadi and Tanha Maafi 2008, Tanha Maafi *et al*. 2007). However, this study has shown for the first time that *H. filipjevi* is also widespread in Khuzestan province and that *H. avenae* is the predominant species in this region.

Distribution of *H. avenae* in Khuzestan province and two other western provinces with common borders with Iraq suggests that it is possible that *H. avenae* could have originally spread from that country into Iran. Although CCN were observed in different wheat cultivars, local barley cultivars and weeds under field conditions, it would be helpful to examine the CCN susceptibility of a fuller range of bread and durum wheat cultivars and weeds under controlled conditions in a glasshouse.

Table 1. Occurrence and population density of cereal cyst nematodes, *Heterodera avenae* and *H. filipjevi,* cysts, eggs and second-stage juveniles (J2) in samples from 124 wheat and 20 barley fields in Khuzestan province, Iran, during 2008-2009.

District	Samples (no)		Infested samples (%)		Cyst density (cysts/100 g soil)		Eggs and J2 (no/100 g soil)	
	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley
Ahvaz	11	1	9	0	30	0	50	0
Andimeshk	7	0	60	0	4	0	70	0
Baghmalek	3	5	0	60	0	48	0	634
Behbahan	7	0	86	0	9	0	375	0
Dasht-e-Azadeghan	13	0	8	0	1	0	100	0
Dezful	10	0	50	0	27	0	456	0
Gotvand	8	0	38	0	6	0	190	0
Hendijan	4	1	50	0	15	0	558	0
Izeh	3	0	33	0	18	0	733	0
Korramshar	5	2	0	0	0	0	0	0
Lali	2	2	100	100	44	41	200	333
Mah Shar	5	0	0	0	0	0	0	0
Masjed Soleiman	2	0	100	0	67	0	483	0
Omidiyeh	4	1	75	100	7	9	240	200
Ramhormoz	6	3	33	33	7	20	266	167
Ramshir	7	0	71	0	15	0	1040	0
Shadegan	5	1	20	0	10	0	500	0
Shushtar	11	3	55	0	11	0	474	0
Susa	11	1	27	0	17	0	576	0
Overall	124	20	37	35	18	27	394	333

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OCCURRENCE OF CEREAL CYST NEMATODE, *HETERODERA AVENAE,* IN SOUTHEAST ANATOLIA, TURKEY*

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SUMMARY

During a survey in 2009, 74 soil samples collected from 27 districts from wheat and barley fields in Southeast Anatolia (Gaziantep, Kilis and Şanlıurfa). Cyst-forming nematodes were only found in 11 samples and were identified as *Heterodera avenae* by PCR–RFLP.

INTRODUCTION

Cereal cyst nematodes cause serious economical damage in cereal crops worldwide especially in temperate regions (Nicol and Rivoal 2008, Rivoal and Cook 1993, Evans and Rowe 1998). *Heterodera avenae* group consists of 12 valid and several undescribed species that infect cereals and grasses (Wouts *et al*. 1995, Gabler *et al*. 2000, Andres *et al*. 2001).

Recent surveys of cereal fields on the Anatolian Plateau of Turkey showed that *Heterodera filipjevi* are widely distributed in major wheat and barley cultivating areas (Yıldırım 2006, Şahin 2009). It was first detected in Turkey during 1995 (Rumpenhorst *et al*. 1996) and being found in 87% of the wheat growing area in the Central Anatolian Plateau (CAP). Yield losses up to 50% were recorded in commonly grown cultivars in CAP in Turkey (Nicol *et al*. 2006). *H. avenae* has been found in Turkey by Yüksel (1973) and Subbotin *et al*. (2003). The information on the occurrence of CCN from other parts of Turkey is limited.

*İmren M, Toktay H, Öcal A, Nicol JM, Elekçioğlu İH (2009) Occurrence of cereal cyst nematode, *Heterodera avenae* in Southeast Anatolia, Turkey. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 82-87. (CIMMYT: Ankara, Turkey)

The objective of this study was to identify species of cereal cyst nematodes from Southeast Anatolia.

METHODS

Surveys of cereal cyst nematodes were conducted over summer in 2009. Samples were taken from 27 sites in the major wheat and barley growing areas in several provinces in the Southeast Anatolian of Turkey. Two kg of randomly sampled soil was collected in each studied field. Soil samples were processed through Kort elutriator (Kort 1960). Extracted cysts caught on the 250-µm-pore sieve were picked with a brush and gathered under a stereomicroscope.

DNA extraction. The total DNA from one or several cysts was extracted using DNA isolation kit (Fermentas Life Sciences).

PCR. 5 µl of extracted DNA was transferred to an Eppendorf tube containing: 2.5 µl 1x Taq incubation buffer, 2 mM of $MgCl_2$ 200 µM dNTPs mixture, 0.4 µM of each primer, 0.2 µl of Taq DNA polymerase and double distilled water to a final volume of 25 µl. The forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT- 3') described by Joyce *et al*. (1994), were used in the PCR. PCR cycles consisted of an initial denaturation step at 94C for 4 min followed by 35 cycles of 1 min at 94C (denaturation), 1.5 min at 60C (annealing), and 2 min at 72C (elongation). The reaction was terminated by a final extension cycle (72C, 10 min), and the PCR product was stored at 4C. The PCR 5 ml of the product was run on a 1% agarose gel and visualised after electrophoresis (120 V) under ultraviolet light (260 nm).

RFLP. Ten microlitres of the PCR product was digested by one of the following restriction enzymes: *AluI*, *Pst* I and *BsuRI (HaeIII*) in the buffer stipulated by the manufacturer (Fermentas). The digested DNA was run on a 2% TAE buffered agarose gel, stained with ethidium bromide and photographed.

RESULTS AND DISCUSSION

The amplification of the ITS region including the flanking parts of the 18S and 28S genes yielded a single fragment of approximately 1200 bp for all studied populations (Figure 1). The information of eleven *H. avenae* populations found in this survey are also given in Table 1.

Eleven populations of *H. avenae* were obviously differentiated by the 566 and 483 bp fragments resulting from *AluI* digestion (Figure 2), 708 and 341 bp fragments resulting from *PstI* digestion (Figure 3), 420 and 353 bp fragments resulting from *BsuRI* (*HaeIII*) digestion (Figure 4).

The RFLP patterns obtained from the studied populations did not reveal any difference with those previously reported for *H. avenae* (Subbotin *et al*. 2003, Mandani *et al*. 2004, Abidou *et al*. 2005). *H. avenae* can be distinguished from the other species by the restriction enzyme *AluI.* This enzyme reveals heterogeneity of the ITS region among several specimens from some populations of *H. avenae*. These RFLP profiles should be considered for identification of populations of this species.

Table 1. Occurrence of *Heterodera avenae* in Southeast Anatolia, Turkey.

Code	Location	Province	Host
21	Elbeyli-Havuzluçam	Kilis	Wheat
28	Elbeyli-Sınır Karakolu	Kilis	Barley
30	Elbeyli-Sınır Karakolu	Kilis	Wheat
31	Elbeyli- Sınır Ardıçlı	Kilis	Wheat
33	Elbeyli- Sınır Ardıçlı	Kilis	Barley
50	Karkamış- Kıvırcık-Sınır	Gaziantep	Wheat
62	Karkamış- Oğuzeli Yolu	Gaziantep	Wheat
65	Karkamış- Akçaköy	Gaziantep	Wheat
66	Karkamış- Akçaköy	Gaziantep	Barley
67	Karkamış- Arıkdere	Gaziantep	Barley
72	Karkamış- Küçükeşme	Gaziantep	Wheat
74	Karkamış- Soylu	Gaziantep	Wheat

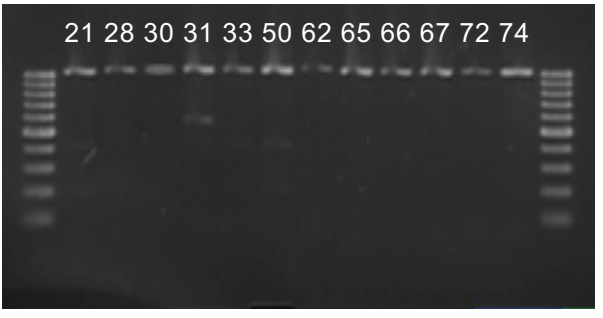

Figure 1. All studied populations yielded a single fragment of about 1200 bp. For species code see Table 1; M, 100 bp DNA ladder.

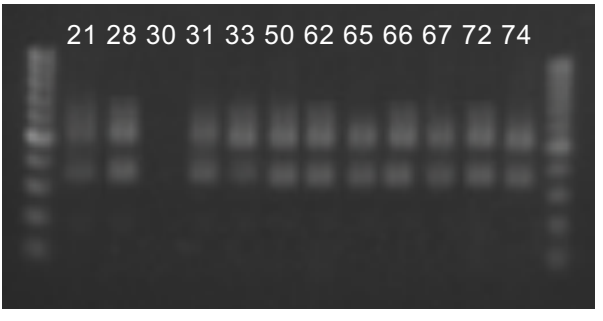

Figure 2. Restriction fragments (566 and 483 bp) of amplified ITS regions of *Heterodera avenae* digested by *AluI*. For species code see Table 1; M, 100 bp DNA ladder.

Figure 3. Restriction fragments (708 and 341 bp) of amplified ITS regions of *Heterodera avenae* digested by *PstI*. For species code see Table 1; M, 100 bp DNA ladder.

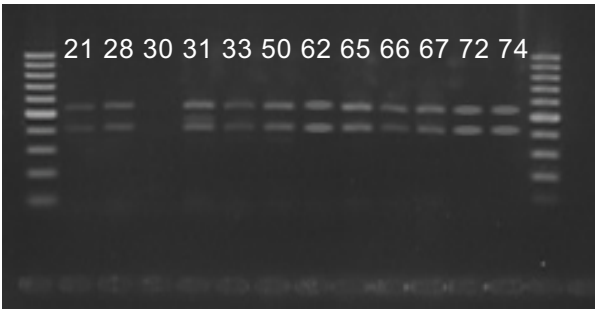

Figure 4. Restriction fragments (420 and 353 bp) of amplified ITS regions of *Heterodera avenae* digested by *BsuRI (HaeIII)*. For species code see Table 1; M, 100 bp DNA ladder.

Other the restriction enzymes *PstI* and *BsuRI (HaeIII)* can be distinguished from the other *Heterodera avenae* group species (Subbotin *et al*. 2003, Mandani *et al*. 2004, Abidou *et al*. 2005).

H. avenae has been reported in Australia, Canada, India, Israel, Japan and most European countries, South Africa and USA (Smiley *et al*. 1994, Nicol and Rivoal 2008). It is also found in Morocco, Tunisia, Pakistan, Libya, Turkey (Rumpenhorst *et al*. 1996), and Estonia (Krall *et al*. 1999). *H.filipjevi* is widely distributed in major wheat and barley cultivating areas (Şahin 2008) and was found in 78% of the wheat growing areas of the CAP. *H. avenae* were reported from Turkey by Yüksel (1973), Subbotin *et al*. (2003) and Abidou (2005) by using only single samples for identifications. Therefore, further comprehensive surveys are needed to more accurately define the distribution of CCN in Turkey.

This study highlights the ecoregional distribution of CCN in Turkey, where *H. filipjevi* is clearly the dominant species on the CAP (the major winter wheat producing area). However, in the predominantly Mediterranean spring wheat area of southeast Turkey *H. avenae* is predominant. More work is needed to map population densities and to define potential economic damage.

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STATUS OF CEREAL CYST NEMATODE IN WHEAT CROPPING SYSTEMS AND RESISTANCE IN PUNJAB, INDIA*

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SUMMARY

Of about 200 fields sampled in Punjab, India (2002-09), 15 showed 100% incidence of CCN with the highest number of cysts (140/250 ml of soil) from Baghapurana (District Moga). Its population was higher in permanent beds in rice-wheat cropping system. About 6,000 lines of wheat and barley were evaluated for resistance. Resistance was identified in 12 lines of wheat from Australia, Mexico and India (AUS15854, AUS15895, two synthetic wheats, KBRL13 resistant to Karnal bunt, W8627, W3339, W9500, W7918, W8697, W8436 and W5793 multiple resistant stock). Seven of 189 accessions of *Aegilops tauschii* and two barley lines (RD2035 and PL718) for the Ludhiana population of the CCN, and three derivatives of KBRL22/3*PBW343 were moderately resistant. Research to understand the genetics of resistance and to devise strategies for incorporating resistance into high yielding cultivars has commenced. Recombinant inbred lines are also being developed for molecular analysis.

INTRODUCTION

The cereal cyst nematode (CCN), *Heterodera avenae* is an important nematode pest of wheat, barley and oat especially in sandy soils. In some areas of India, infestation has resulted in complete crop failure (Van Berkum and Seshadri 1970*).* Whereas in the 1990s in Punjab, *H. avenae* populations remained below a damaging threshold in rice-wheat rotations. Since 2003, damaging infestations of CCN have been found in the fields with a rice-wheat rotation. The nematode can be managed by cultural practices, chemicals and using CCN resistant cultivars or integrating these methods. Resistance is the most effective option for CCN management. Resistance in wheat

*Kaur DJ, Sharma I, Sohu VS, Bains NS, Singh Y (2009) Status of cereal cyst nematode in wheat cropping systems and resistance in Punjab, India. In 'Cereal cyst nematodes: status and research.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 88-93. (CIMMYT: Ankara, Turkey)

has been widely reported but relatively few CCN resistant cultivars are grown commercially (Nicol *et al.* 2001). Only one cultivar (Raj MR-1) has been recommended for cultivation in the Indian states of Rajasthan and Haryana (Bhatti and Dahiya 1992, Sharma and Sharma 2000). The Ludhiana population of the nematode is different from those of Haryana, Delhi and Rajasthan and has been taxonomically determined as *H. filipjevi* (Bishnoi *et al.* 2004). The cultivars resistant to CCN from other states have proven to be susceptible to the Ludhiana population. In Punjab, the predominant cultivar, PBW 343, is susceptible to CCN, hence it is vital to identify resistance to both types of CCN and to develop high yielding resistant cultivars acceptable for commercial deployment.

METHODS

Monitoring. Soil and root samples were collected and analysed for nematode cysts. Effect of agronomic practices on CCN was studied in rice-wheat cropping system.

***Screening*.** Wheat and barley genotypes from multilocation advanced varietal trials, synthetic wheat lines obtained from CIMMYT, multiple disease resistant lines and *Aegilops tauschi* accessions were screened in inoculated pots and in CCN-infested field plot. The data were recorded on white females/plant and the lines were rated on a 5-point scale. For confirmation, entries resistant to CCN were retested in subsequent years.

***Genetic basis of resistance*.** Inheritance of resistance to CCN in wheat was studied by crossing high yielding wheat line, PBW343, with lines, AUS15854, W8627 and synthetic wheat 19-1, all showing strong resistance to CCN.

RESULTS

Monitoring. Eighty eight of the 738 samples collected from about 200 fields contained CCN infested wheat roots at a frequency of 17 to 100% between specific localities and seasons (Table 1). Cysts numbers were particularly high in the samples collected from Baghapurana and Raikot (District Moga and Ludhiana). The crops exhibited symptoms of the nematode infestation at Baghapurana. Cysts were also observed in some crops in rice-wheat rotation. In rice-wheat cropping system, the CCN population densities were higher in wheat on permanent beds in comparison to other agronomic practices.

Resistant stocks. About 5,000 lines of wheat and 1,000 lines of barley were screened over the last 15 years. Resistance to CCN was recorded in twelve lines of wheat and two of barley (Table 2). Of the lines reported as resistant, only two, AUS15854 and AUS15895, showed resistance to CCN populations evaluated. Among the synthetic wheat 19-1 and 63 showed resistance to CCN, Karnal bunt, stripe and leaf rusts. Similarly, seven of 32 bread wheat lines with multiple resistance to loose smut, leaf and stripe rusts, and Karnal bunt were found to be resistant to CCN (*viz.* W3339, W5793, W8627, W9500, W8697, W8436 and W7918). Another Karnal bunt resistant stock (KBRL13) and seven of 189 *A. tauschii* accessions showed resistance to CCN (*viz.* AT104, AT138, AT186, AT264, AT270, AT272 and AT282). In barley, only two lines (RD2035 and PL718) showed resistance. In the material under advanced varietal trials (AVT) though resistance was not found but a number of

lines showed moderate resistance (*viz.* wheat lines HPW296, HPW308, PBW621, HS463, MPO1220(D), NIAW1415, HPW286, DWR28A, PDW312(D), SWL26, AKDW 2997-6, MP4106, SKW323, PDW306, WH 1025, PDW 304, HW 1095 HS463, VL878, HI8645, HW5041, UP 2596, MACS2496, K0243 and DDK1028, and barley lines PL802, BHS352, BHS169, DWR46, RD2624, RD2677, NDB 1289, Carina/Salmas, VA93-42-23, WreselburgerAhor1303-61/3Arr/Esp//Alger/Ceres362-1-1 and Moroc9-75/SLB39-60).

Table 1. *Heterodera avenae* infestation on wheat in Punjab (2002-09).

Year	Samples collected	Samples infested (*H. avenae* cysts/250 ml soil+roots)			
		No.	Village/Locality	Cysts	Freq. (%)
2002-03	128	24	Bagha Purana	20-140	50
			Raikot	31-119	100
			Moga, Ghall Kalan, Bopara, Maler Kotla and Vajidke	2-10	50-100
2003-04	73	12	Punjgrahian	10-50	100
			Kotkapura, Ludhiana	2-11	100
2004-05	118	18	Chanauli	42-59	100
			Machhiwara	40-60	100
			Barwa, Dharamkot, Ludhiana	2-21	100
2005-06	36	0		-	-
2006-07	134	21	Gidarbaha	11-20	100
			Handyaya, Karimpura, Salora	1-10	50-100
			Mandiala and Malikpur		
2007-08	26	9	Mahal Kalan	28-42	40
			Kotkpura, Gobindgarh, Khara	4-9	17-50
2008-09	123	4	Ropar, Naushehra, Nurpura	2-4	25-50
Total	738	88		1-140	17-100

Genetic basis for resistance. Efforts undertaken to understand genetics of resistance have been presented in Tables 3 and 4. Recombinant inbred lines are being developed from the cross synthetic 19-1 x PBW343 and plant population from F5 was evaluated for CCN resistance during 2008 in both CCN-inoculated pots and field conditions. PBW343 was planted as check. Of 48 recombinant inbred lines (RILs) all the 7 plants from 5 RILs showed susceptibility comparable to the susceptible check. Resistance was observed in 43 RILs indicating 2 genes governing resistance which is confirmed by the χ^2 value. The lines are being further advanced to F6 in a summer nursery planted at Dalang Maidaan, Himachal Pradesh. F6 RILs will be planted in both pots and fields with CCN infestation in the forthcoming season. We understand that number of RILs are relatively small for such an analysis, however it is being presented here as only few RILs showed susceptibility compared

to the susceptible check under both pot and field conditions, providing strength to our observation. Three CCN resistant genetic stocks (AUS15854, synthetic 19-1 and W8627) were again crossed with PBW343, both to determine the number and nature of genes. Seeds of individual F1 plants were harvested to provide an F2 generation for further development of RILs for confirmation of genetics of resistance and molecular analysis. In F1 generation of all the three crosses, the plants showed 4-5 females per plant, whereas the resistant parent showed <4 females and susceptible parent >12 females indicating more skewing towards dominance of resistance in the crosses evaluated during 2008-09.

Table 2. Wheat and barley entries resistant to cereal cyst nematode (CCN).

Entries	Source	Resistant to CCN
WHEAT		
Advanced Varietal Trial I and II	DWR, Karnal	-
State Trial	PAU, Ludhiana	-
Multiple Disease Screening Nursery	DWR, Karnal	-
Synthetic Wheat	CIMMYT	19-1, 63
CCN resistant lines	RRS, Durgapura	AUS15854, AUS15895
Karnal Bunt Resistant Stocks	PAU, Ludhiana	KBRL13
Karnal bunt resistant lines derived from cross KBRL22/3*PBW 343	PAU, Ludhiana	-
Multiple Disease Resistant Stocks	PAU, Ludhiana	W3339, W8627, W9500, W7918, W8697, W8436, W5793
Aegilops tauschii accessions	PAU, Ludhiana	AT104, AT138, AT186, AT264, AT270, AT272, AT272
BARLEY		
Advanced Varietal Trial I and II	DWR, Karnal	RD2035, PL718
State Trial	PAU, Ludhiana	-

Table 3. Nature of genes in a recombinant inbred population derived from synthetic wheat 19-1 x PBW343.

RILS	Resistant RILs	Susceptible RILs	Genes postulated	Expected ratio	χ^2 value	P value
Observed	43	5	2	7:1	0.0478	0.827
Expected	42	6				

Table 4. Inheritance of resistance for cereal cyst nematode in wheat

F1	MR[1]/total plants	Parent	R/total plants	Gene
AUS15854 x PBW343	6/6	AUS15854	7/7	Dominant
W 8627 x PBW343	7/7	W8627	7/7	Dominant
Synthetic 19-1 x PBW343	6/6	Synthetic 19-1	7/7	Dominant
-	-	PBW343	0/7	Susceptible[2]

[1]4-5 females/plant; [2]>12 females/plant; MR, moderately resistant; R, resistant.

DISCUSSION

H. avenae, which was considered not to be of concern in rice-wheat cropping system in the Punjab state, is not only surviving in some of these fields but also increasing in population density. If this nematode goes on multiplying and spreading in wheat fields, it will have significant impact on the crop. The resistance in the present study has been indicated to be governed by 2 dominant genes in the 3 parental lines (AUS15854, W8627 and synthetic 19-1). Similar to these findings, multiple resistance in wheat to different diseases and nematode pests has been reported in several lines at CIMMYT (Singh and Rajaram 2002).

Resistance has also been reported in *Triticum aestivum* (cv. Loros, AUS10894), *Triticum durum*, barley (cv. Morocco), triticale, oats (*Avena sterilis*) and *A. tauschii* to different pathotypes of *H. avenae* (Eastwood *et al*. 1994, Nicol 2002). In an extensive study of Nicol *et al*. (2007) focused on the the management of soil borne pathogens in wheat and identified/utilised more than 30 sources of resistance in national and international breeding program against CCN, root lesion nematode and crown rot. Resistance in AUS15854 to different populations of *H. avenae* was reported earlier by Mathur and Dalal (1995) and Sharma and Sharma (2000) from Rajasthan. A single dominant gene controlling the CCN resistance in wheat genotype, AUS15854 crossed with Raj3077 has been reported by Sharma and Sharma (2000). Population variation as well as the different susceptible parent involved in the crosses explains the variance in the results. Under the All India Coordinated Project, resistance to CCN has been reported in a few advanced breeding lines only against respective nematode population (Anon. 2003-2008).

Nine CCN resistance genes have been identified in wheat and its relatives, some of which confer resistance to the Australian pathotype of CCN (Ha13). Cultivars released in Australia with CCN resistance carry either the *Cre1* or *Cre8* gene, with the *Cre3* gene present in advanced breeding lines (Ogbonnaya *et al.* 2001). Multiple resistant stock, W8627 identified in the present study will be involved for incorporating resistance in high yielding bread wheat and for the development of RILs.

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DISCOVERY OF *HETERODERA FILIPJEVI* ON WHEAT IN THE USA*

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SUMMARY

Until recently, cyst nematodes from wheat and barley fields in the Pacific Northwest (PNW) of the USA were identified as homogeneous populations of *Heterodera avenae*. During February 2008, while using PCR-RFLP to examine *H. avenae* collected in the PNW, cysts from a winter wheat field exhibiting patches with up to 90% plant mortality near Imbler (Union County, Oregon) consistently revealed a restriction pattern matching that of a *H. filipjevi* DNA standard rather than *H. avenae* with six endonucleases *Taq*I, *Hinf*I, *Pst*I, *Hae*III, *Rsa*I and *Alu*I. The pattern was different from those of *H. latipons* and *H. schachtii* with *Taq*I, *Hae*III and *Rsa*I. Comparisons of cyst vulval cones revealed a characteristic morphological difference between the Imbler cysts and specimens of *H. avenae*. A distinct underbridge with bifurcated arms was present in vulval cones of the Imbler cysts whereas no underbridge was found in *H. avenae*. Other morphological and morphometric characters in cysts and second-stage juveniles were consistent with the description of *H. filipjevi*. Our molecular tests led to the first discovery and documentation of *H. filipjevi* in North America. Grid soil sampling revealed that *H. filipjevi* was present at most of infested grid sites but mixtures of *H. avenae* and *H. filipjevi* also occurred in the field where *H. filipjevi* was discovered. Intraspecific polymorphism was not observed within *H. filipjevi* populations based on ITS-rDNA. The pathotype and effective resistance genes for introgression into wheat are being identified.

INTRODUCTION

The cereal cyst nematodes, *Heterodera avenae* group, contain at least 12 species that invade roots of cereals and grasses. *Heterodera avenae*, *H. filipjevi* and *H. latipons* are recognised as the most economically important of these species. *H. avenae* has wide distribution in temperate wheat-producing regions throughout the world. It was first detected in USA during 1974 and is now known to occur in seven western states (ID, CA, CO, MT, OR, UT, WA) (Smiley *et al*. 2005). Wheat yields were negatively

*Yan GP, Smiley RW (2009) Discovery of *Heterodera filipjevi* on wheat in the USA. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 94-99. (CIMMYT: Ankara, Turkey)

correlated with initial densities of *H. avenae*, with yields of intolerant cultivars in commercial fields being reduced by as much as 50% in Oregon (Smiley *et al*. 1994). *H. filipjevi* is found in eastern and northern Europe, Central and west Asia, Middle East, Indian subcontinent, and North America. *H. latipons* occurs mainly in the Mediterranean region but also in Asia and Europe (Smiley and Nicol 2009).

The use of wheat cultivars that are both resistant and tolerant offers the most effective, economic and environmentally friendly option to control damage. Individual wheat cultivars differ in their ability to resist these nematodes; a cultivar exhibiting resistance to one species was not necessarily resistant to another species. Therefore, optimal cultivar selection requires that the nematode species present in each field or region be accurately identified.

It is challenging to differentiate species in the *H. avenae* group using traditional microscopic method due to difficulties in distinguishing key diagnostic features and great variability of individual specimens within a population. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and internal transcribed spacer (ITS)-rDNA have been used to differentiate these species. The objective of this study was to identify and confirm the *Heterodera* species present in Pacific Northwest (PNW) dryland wheat and barley fields.

METHODS

Soil samples were collected from wheat and barley fields thought to be infested with cereal cyst nematodes near St. Anthony, Idaho; Union, Imbler and Island City (Union County), Oregon; and Palouse, Washington. Cysts were hand-picked from roots and moist soil under a microscope. Three to eight cysts were broken open and smashed for DNA extraction using a commercial kit (Bio 101 FastDNA Kit) or the method described by Rivoal *et al.* (2003). Control DNA of *H. avenae* (IB), *H. filipjevi* (E84 and E88), *H. latipons* (E99, E123 and E156), and *H. schachtii* (Hs) were obtained from cooperators in France. PCR reactions of 20 µl contained 3 µl of the DNA template, 1x PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 0.5 mM $MgCl_2$ and 1.25 units of *Taq* polymerase (Roche, Mannheim, Germany). Primers 18S (5'-TTGATTACGTCCCTGCCCTTT-3') and 26S (5'-TTTCACTCGCCGTTACTAAGG-3') as described by Rivoal *et al*. (2003) were used to amplify the ITS region of rDNA. PCR amplification was performed in a MyCycler™ Thermal Cycler (Bio-Rad, Richmond, CA) as follows: 94C for 2 min followed by 40 cycles of 94C for 1 min, 60C for 50 s, 72C for 1 min, with a final extension at 72C for 7 min. Six restriction endonucleases *Taq*I, *Hinf*I, *Pst*I, *Hae*III, *Rsa*I and *Alu*I (Roche) were used. Digestions were carried out in 15 µl reaction mixtures containing 2 units of the enzyme, 1x enzyme buffer, and 10 µl of the PCR product at optimum incubation conditions as recommended by the manufacturer for each enzyme. The species of *Heterodera* was determined by comparing the restriction pattern with those of the control species in agarose gels by electrophoresis. Examination of cyst and juvenile characteristics was made with a compound light microscope to confirm identification.

RESULTS

RFLP analysis of the PCR products with *Taq*I revealed that the banding patterns from St. Anthony, Palouse, Union and Island City were the same as that of the known control for *H. avenae*, and were different from those of *H. filipjevi*, *H. latipons* and *H. schachtii* (Figure 1). The cysts from these sites were therefore identified as *H. avenae*. However, the cysts from Imbler, Oregon revealed a PCR-RFLP profile matching *H. filipjevi* rather than *H. avenae, H. latipons* and *H. schachtii* (Figure 1).

Digestion with five other endonucleases consistently showed that the samples from Imbler produced the same PCR-RFLP banding pattern as that of *H. filipjevi* but different from that of *H. avenae* (Figure 2). These cysts also produced banding patterns different from those of the control species *H. latipons* and *H. schachtii* when digested with *Hae*III and *Rsa*I, ruling out the possibility that the sample from Imbler could be *H. latipons* or *H. schachtii*.

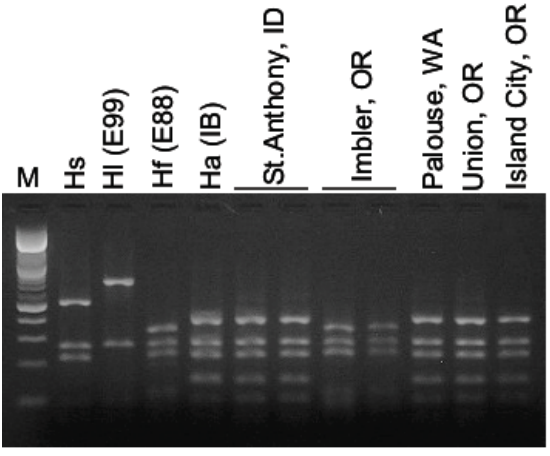

Figure 1. PCR-RFLP profiles of cyst nematodes from fields in the Pacific North West of the USA using *Taq*I enzyme. Hs (*H. schachtii*), Hl (*H. latipons*), Hf (*H. filipjevi*) and Ha (*H. avenae*) are the control species. Tested samples were from St. Anthony, ID; Imbler, Union and Island City, Oregon; and Palouse, Washington. M is the 100-bp DNA ladder.

Microscopic comparisons of cyst vulval cones from the Imbler site and known specimens of *H. avenae* revealed a characteristic difference in the underbridge structure (Figure 3), indicating that the Imbler cysts were not specimens of *H. avenae*. Measurements of second-stage juveniles and cysts support the hypothesis that the cysts from Imbler are *H. filipjevi*, a cereal cyst nematode not previously reported in North America (Smiley *et al*. 2008).

Grid soil sampling was conducted in the Imbler field where *H. filipjevi* was detected. Cyst nematodes were not detected in 35 of 50 sampling sites. Of the 15 grid sites infested with cyst nematodes, 12 sites contained only *H. filipjevi* and 3 sites contained mixtures of *H. avenae* and *H. filipjevi*. The RFLP banding patterns with

*Taq*I are shown in Figure 4 for three of the 12 sites with only *H. filipjevi* and one of the three sites with mixtures of *H. avenae* and *H. filipjevi*. Intraspecific polymorphism was not observed within the *H. filipjevi* populations using *Rsa*I and *Alu*I digestions.

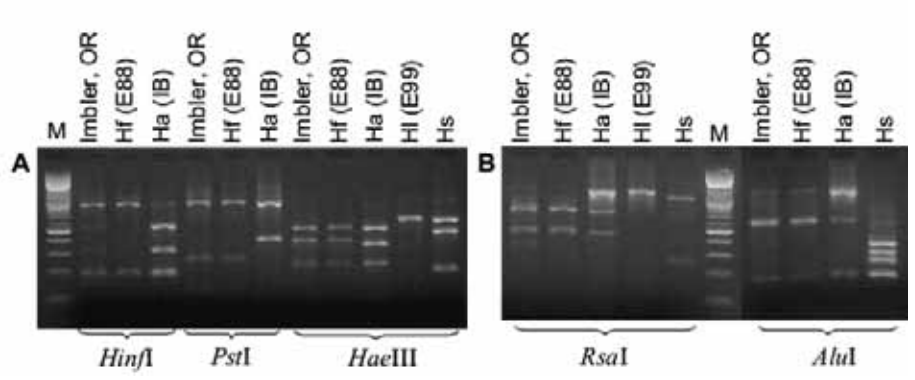

Figure. 2. PCR-RFLP patterns for the sample from Imbler, OR, USA digested with five restriction endonucleases. **A:** *Hinf*I, *Pst*I and *Hae*III. **B:** *Rsa*I and *Alu*I. Hf (*H. filipjevi*), Ha (*H. avenae*), Hl (*H. latipons*) and Hs (*H. schachtii*) are the control species. M is the 100-bp DNA ladder.

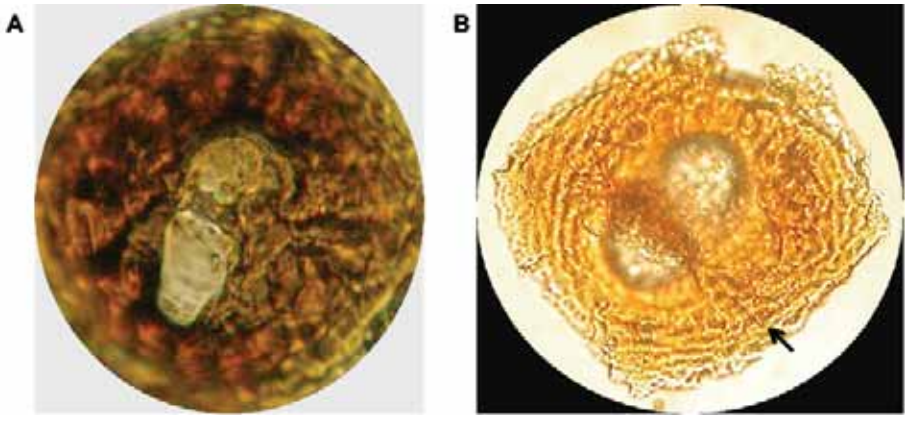

Figure 3. Microscopic views of vulval cones from cysts of *Heterodera*; images are approximately 40 µm in diameter. **A:** fenestral area without an underbridge from a cyst of *H. avenae*. **B:** fenestral area with a distinct underbridge (arrow) characteristic of species of *H. filipjevi* from an Imbler cyst.

DISCUSSION

PCR-RFLP tests with each of six endonucleases allowed for rapid discrimination of *H. filipjevi* and *H. avenae* cysts extracted from PNW wheat fields. *H. filipjevi* and *H. avenae* were readily distinguished from *H. Latipons* and *H. schachtii* (a widely distributed beet cyst nematode in Idaho and Oregon) with three of the endonucleases. Furthermore, key morphological difference between *H. filipjevi* and *H. avenae* were obtained to confirm the accuracy of the species identification

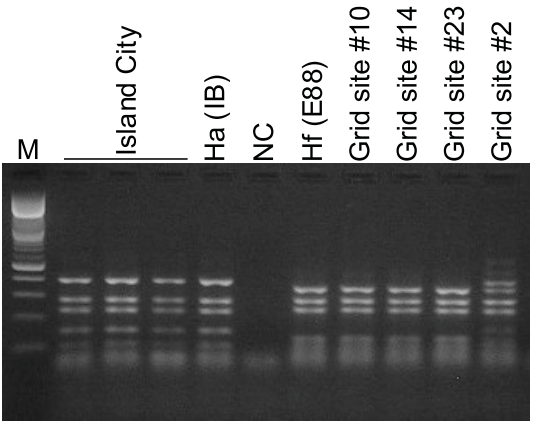

Figure 4. PCR-RFLP profiles of cyst nematodes from fields in Island City, Oregon, USA and grid sampling sites #2, #10, #14 and #23 (Imbler, Oregon) using *Taq*I enzyme. Ha (*Heterodera avenae*) and Hf (*H. filipjevi*) are the control species. NC represents negative control without any nematode DNA template. M is the 100-bp DNA ladder.

determined by PCR-RFLP. Generally, only one species in the *H. avenae* group is identified in most regions but mixtures of species can also occur within individual fields (Smiley and Nicol 2009). Our tests revealed that *H. filipjevi* was the only *Heterodera* sp. present at most of the infested sites in the field that was grid-sampled. Mixtures of *H. avenae* and *H. filipjevi* were also detected at several other grid sites.

The cyst nematodes in soils collected from most areas of Union County, Oregon and two locations in Washington and Idaho were identified as *H. avenae* using the PCR-RFLP technique. To date two reports have applied biochemical and molecular procedures to PNW populations of *H. avenae*. Ferris *et al*. (1994) reported that one isolate from Oregon and one from Idaho differed on the basis of 2-D PAGE protein patterns, but that both isolates showed protein patterns consistent with the species concept for *H. avenae*. Subbotin *et al*. (2003) compared the rDNA ITS sequence data of these two *H. avenae* isolates with consensus sequence data of *H. avenae* from other regions and stated, without presentation of data, that the two North American isolates clustered with European *H. avenae*. Populations from three *H. avenae*-infested farms in Union County, Oregon were also reported as *H. avenae* based on upon commercial but proprietary DNA tests provided by South Australian Research and Development Institute, Adelaide, Australia (Ophel-Keller, 2008). However, the Australian test was not designed to distinguish among species of the *H. avenae* group (A. C. McKay, personal communication).

The use of resistant cultivars offers the most effective and economic option to control damage from cereal cyst nematodes. The *Cre1* gene carried by a hexaploid wheat line that is not adapted to the PNW climate was shown to be capable of preventing reproduction of *H. avenae* populations in eastern and western Oregon (Smiley 2009). The *Cre1* gene has been incorporated into PNW-adapted wheat cultivars. Effective resistance genes against *H. filipjevi* in Oregon are being identified using the International Test Assortment composed of 23 barley, wheat and oat lines containing defined genes for resistance to members of the *H. avenae* group

including *H. filipjevi*. The best possible outcome would be a demonstration that the *Cre1* gene is as effective against *H. filipjevi* as it is against *H. avenae* in Oregon. If the Oregon population of *H. filipjevi* is not controlled by *Cre1*, another gene identified from our tests will be introduced into the PNW wheat cultivars already carrying the *Cre1* gene, pyramiding these sources of resistance to both nematodes. This will be important in fields where *H. filipjevi* already coexists with *H. avenae*, and in *H. avenae*-infested fields likely to also become infested with *H. filipjevi* due to the efficient spread of these species in soil transferred by wind, water, and contamination by soil of agricultural products, animals, equipment and humans (dusty or muddy boots or clothing).

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FREQUENCY AND DIVERSITY OF CEREAL NEMATODES ON THE CENTRAL ANATOLIAN PLATEAU OF TURKEY*

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SUMMARY

Distribution of important plant parasitic nematodes on Central Anatolian Plateau in Turkey was investigated by systematic surveys of cereal fields conducted in between 2003 and 2007. Cereal cyst nematode was found in 78% and root lesion nematode in 43% of soil samples. Cereal cyst nematode in all provinces were identified as *Heterodera filipjevi*, with *H. latipons* found in one province only. *Pratylencus thornei* and *P. neglectus* were the most widely distributed species of root lesion nematode in surveyed area. *Pratylencus loosi*, *P. scribneri* and *P. crenatus* were also recorded in some samples. Other important plant parasitic nematodes were *Pratylenchoides* sp. (36%), *Geocenamus* sp. (52%), *Ditylenchus* sp. (62%) and tylenchid nematodes (37%).

INTRODUCTION

Turkey is one of the ten largest wheat producers in the world with gross production of 21 Mt and average yield of 2.3 t/ha (Anon. 2009a). Thirty five per cent of the total 9 Mha is under rain-fed winter wheat production on the Central Anatolian

*Şahin E, Nicol JM, Elekçioğlu İH, Yorgancılar Ö, Yıldırım AF, Tülek A, Hekimhan H, Yorgancılar A, Kılınç AT, Bolat N, Erginbaş-Orakcı G (2009) Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 100-105. (CIMMYT: Ankara, Turkey)

Plateau (CAP) where drought stress is common. Yield loses become severe when drought occurs along with other root disease and pests.

Plant parasitic nematodes are one of the important biotic constraints on cereal production in Turkey. Lung (1992) and Rumpenhorst *et al.* (1996), reported the presence of cereal cyst nematode and root lesion nematodes in CAP. Elekçioğlu (1996), gave first reports of *Geocenamus hexagrammus, G. rugosus* and *Pratylenchoides laticauda* for Turkey and *Pratylenchoides leiocauda* for South Mediterranean region of Turkey. Abidou *et al.* (2005) have investigated the distribution of cereal nematodes in CAP and Mediterranean region in Turkey. However, studies were very local and with insufficient detailed of the distribution of cereal nematodes in the rain-fed winter wheat production system of the Central Anatolian Plateau.

METHODS

Sampling Locations

A total of 286 locations were sampled systematically in 2003, 2004 and 2005. Soil samples were collected from cereal fields by stopping at random every 10 km along the roadside. Soil samples were taken at the early stage of the cropping season (March-April) to determine motile and sedentary nematodes. After evaluation of results from this initial survey, a further 117 soil samples were collected in June 2007 from districts on CAP (Figure 1) with high cereal cyst nematode population densities to provide fresh full cysts for molecular identification. One of 2 kg bulk sample was taken consisting of 15 subsamples from a 5 ha field area at each location. Each subsample was collected by using a 2 cm diameter auger to a depth of 20 cm.

Figure 1. Provinces of the Central Anatolian Plateau, Turkey sampled for cereal nematodes (indicated by a star symbol).

Nematode Extraction and Identification

Migratory nematodes were extracted using the modified Whitehead tray technique from 200 g of each soil sample (Hooper 1986, Whitehead and Hemming 1965). All

nematodes were identified to genus level based on key morphological features. Identification of the specimens was performed in the basis of the morphometric and morphologic characters using relevant references for each genus. Cysts were extracted using the modified Fenwick can method (Stirling 1999) from 250 g soil sample. The number of cysts with or without eggs for each sample was counted. Cyst nematodes were identified in basis of the vulva and second stage juvenile morphometrics and morphological characters using relevant references and also using molecular PCR-RFLP technique (Bekal *et al.* 1997, Subbotin *et al.* 1999, Abidou *et al.* 2005).

RESULTS

Frequency and Distribution of plant Parasitic Nematodes from 2003-2005

Plant parasitic nematodes were present in 85% of soil samples. *Heterodera* cysts were found in 78% of samples and 43% of samples contained *Pratylenchus* species. Cereal cyst nematode cysts and root lesion nematodes were found together in same location in 34% of sampling areas. *Pratylenchoides* sp. (36%), *Geocenamus* sp. (52%), *Paratylenchus* sp. (19%), *Helicotylenchus* sp. (30%), *Trophurus* sp. (6%), *Tylenchorinchus* sp. (5%), *Ditylenchus* sp. (62%) and *Tylenchus* sp. (37%) were other plant parasitic nematodes found in soil samples.

Population Density and Species Identification of Key Plant Parasitic Nematodes

Cereal cyst nematodes were identified as *Heterodera filipjevi* in all locations, other than one location in Yozgat where *H. latipons* was found. High population densities (4-41 cysts/250 g dry soil) of cereal cyst nematode were recorded in almost all locations as presented in Table 1. Considering the average number of eggs per cyst conservatively would be 150, the range of number of eggs or juveniles of *H. filipjevi* would be 3-30/g of soil. Economic density for yield loss is considered to be in the range of 5 eggs/g soil (unpublished data). Considering other plant parasitic nematodes, *Pratylenchus thornei* and *P. neglectus* are the most widely distributed root lesion nematode species in surveyed areas, with *P. thornei* being recorded in 10 provinces and *P. neglectus* in 8 provinces. Other *Pratylenchus* species identified included *P. loosi* and *P. scribneri* (one province only) and *P. crenatus* (2 provinces) (Table 1).

As listed in Table 1, other plant parasitic nematodes included the following species; *Geocenamus brevidens, G. microdorus, Pratylenchoides alkani, P. erzurumensis, Scutylenchus rugosus*, *S. tumensis*, *Trophurus impar*, *Tylenchorhynchus mamillatus T. striatus* and *Zygotylenchus guevarai*,

DISCUSSION

Cereal cyst and root lesion nematodes are considered the most important plant parasitic nematodes for wheat production and were found widely distributed in CAP in Turkey. Cereal cyst nematode being found in 78% of the surveyed areas was identified as *H. filipjevi* except for one population from Yozgat identified as *H. latipons*. Rumpenhorst *et al.* (1996) reported cyst nematodes of the *H. avenae* group in 41% of samples from the CAP, Turkey. They found the majority of samples were *H. filipjevi,* but also found *H. avenae* at one site and *H. latipons* at two sites. They

Table 1. Cereal cyst nematode (CCN, cysts/250 g soil) and *Pratylenchus* spp. (no/200 g soil) populations densities and species of other plant parasitic nematodes in soil sampled from 16 provinces of Turkey in 2003-2005; CCN identified as *Heterodera filipjevi* in 15 provinces and as both *H. filipjevi* and *Heterodera latipons* in Yozgat.

Province	Samples	CCN[1]	*Pratylenchus*		Other plant parasitic nematodes
			Species	Density[2]	
Afyon	17	10 (0-42)	-	9 (0-60)	-
Ankara	29	14 (0-73)	*P. crenatus* *P. loosi* *P. thornei*	21 (0-130)	*Pratylenchoides alkani, Pratylenchoides erzurumensis, Scutylenchus rugosus, Tylenchorhychus striatus*
Bilecik	7	7 (0-16)	*P. thornei*	21 (0-170)	*P. alkani, P. erzurumensis, S. rugosus*
Bolu	15	6 (0-42)	*P. crenatus* *P. neglectus* *P. scribneri* *P. thornei*	11 (0-40)	*P. alkani, S. rugosus, Zygotylenchus guevarai*
Çorum	20	5 (0-35)	*P. thornei*	9 (0-80)	*Trophurus impar*
Denizli	16	4 (0-23)	*P. thornei*	30 (0-120)	*Geocenamus brevidens, P. alkani,* *P. erzurumensis, Scutylenchus tumensis*
Eskişehir	17	22 (0-95)	-	27 (0-100)	-
Isparta	7	16 (2-71)	-	11 (0-80)	*G. brevidens, P. alkani*
Kayseri	19	14 (0-39)	*P. neglectus*	33 (0-130)	*S. rugosus*
Kırıkkale	7	15 (0-86)	*P. neglectus* *P. thornei*	120 (0-380)	-
Kırşehir	6	41 (12-89)	*P. neglectus*	53 (0-220)	*S. rugosus, Tylenchorhynchus mamillatus*
Konya	49	12 (0-61)	*P. thornei*	40 (0-520)	*G. brevidens, Geocenamus microdorus, P. erzurumensis*
Niğde	11	23 (0-94)	*P. neglectus* *P. thornei*	92 (0-430)	*S. rugosus*
Sivas	20	15 (0-73)	*P. neglectus*	34 (0-380)	-
Uşak	11	9 (0-61)	*P. neglectus* *P. thornei*	20 (0-100)	*G. microdorus, P. alkani*
Yozgat[3]	17	25 (0-212)	*P. neglectus* *P. thornei*	9 (0-60)	*G. microdorus, S. rugosus*

[1]Mean (range) cysts/250 g of soil. [2]Mean (range) numbers/200 g of soil. [3]Both *H. filipjevi* and *H. latipons* occur.

also report species mixtures in two samples. Abidou *et al.* (2005) also reported 85% incidence of cyst nematodes belonging to *H. filipjevi* and *H. latipons* as pure or mixed species in locations with high populations on the CAP.

Root lesion nematodes also appears to be of importance in Turkey wheat cropping areas. As with the global literature, the most frequently recorded species in this study were *Pratylenchus thornei* and *P. neglectus*. Abidou *et al.* (2005) found about 40% of soil samples infected with *P. thornei* and/or *P. neglectus* in CAP, Turkey. These two species were commonly found together in the present study. Other *Pratylenchus* species reported included *P. loosi, P. scribneri* and *P. crenatus*. Wheat is reported as weak host for *P. scribneri* and *P. crenatus* and a non-host for *P. loosi* (Rich *et a.* 1977, Anon. 2009b,c), and hence these species are probably not of great economic importance to cereals.

Pratylenchoides sp., *Geocenamus* sp., *Ditylenchus* sp. and tylenchid nematodes was the most predominant other plant parasitic nematodes. *Pratylenchoides ritteri* and *Zygotylenchus guevarai* were also reported in France, Italy and Spain to be important in wheat (Griffin 1984). Smiley *et al.* (2006) also reported damage by *Geocenamus brevidens* to wheat in Oregon.

It is clear from this study that cereal cyst nematode (*H. filipjevi*) and the two root lesion nematode species (*P. thornei* and *P. neglectus*) have a widespread distributions in the rain-fed wheat cropping zone of the Anatolian Plateau in Turkey. *H. filipjevi* would be damaging in many cases, according to our estimates of population densities, and probably the two species of root lesion nematode (*P. thornei* and *P. neglectus*) as well. Further investigation is also suggested for *Pratylenchoides* sp., *Geocenamus* sp., *Paratylenchus* sp. and *Helicotylenchus* sp. where population densities appeared high.

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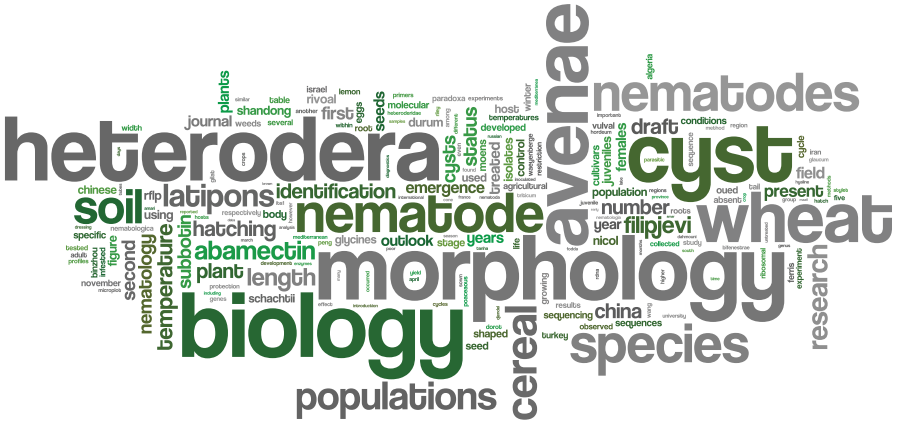
PART 3 MORPHOLOGY AND BIOLOGY



MOLECULAR IDENTIFICATION OF *HETERODERA* SPP., AN OVERVIEW OF FIFTEEN YEARS OF RESEARCH*

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SUMMARY

During the last 15 years, researchers have collected and characterised more than 40 species of nematodes from the genus *Heterodera*. The species were identified by sequencing the ITS–rRNA genes and by PCR-RFLP profiles; these tools remain the best available for identifying cyst-forming nematodes. By restricting the ITS amplicons with one or a combination of seven restriction enzymes (*Alu*I, *Ava*I, *Bsh*1236I, *Bsu*RI, *Cfo*I, *Mva*I, and *Rsa*I), researchers can distinguish most of the agriculturally important cyst nematode species from one another and from their sibling species. Species from the Avenae group can be differentiated from one another using the enzymes *Alu*I, *Cfo*I, *Hinf*I, *Ita*I, *Pst*I, *Rsa*I, *Taq*I and *Tru*9I. However, in some cases, it is not possible to use sequences of ITS–rRNA genes and PCR-RFLPs in diagnostic work. In these cases, morphometric characteristics are better for differentiating these species. Intraspecific polymorphism in the ITS sequences can make identification even more difficult; here, more conclusive molecular identification tools are needed to diagnose some species. In the future, end-point PCR and semi-quantitative PCR (SYBR Green I) with species-specific primers (already developed for *Heterodera glycines* and *H. schachtii)* will be the likely choices for fast and reliable detection and quantification of cyst nematodes in samples.

*Waeyenberge L, Viaene N, Subbotin SA, Moens M (2009) Molecular identification of *Heterodera* spp., an overview of fifteen years of research. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 109-114. (CIMMYT: Ankara, Turkey)

INTRODUCTION

The genus *Heterodera* contains more than 60 species, some of which cause serious yield reduction in crops. Beet crops are intensely affected by *Heterodera schachtii,* and many species of cereal cyst nematodes reduce grain harvests worldwide. The protective cyst stage of these nematodes enables them to withstand desiccation and greatly enhances their dispersal and survival. Rapid and reliable identification of nematodes intercepted by phytosanitary authorities is an important step in monitoring and controlling the movement or introduction of potential pests. Some nematodes are of regulatory concern, and as international trade expands, vigilance and accurate diagnosis become even more important to prevent their dispersion. Application of control measures, especially when growing resistant crops, requires accurate identification of the cyst nematode up to species level.

To date, morphological identification posed particular problems, with many isolates not reliably identifiable to species level. In the last 15 years, polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) profiles and sequences of the internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal RNA genes of more than 40 different *Heterodera* spp. have been developed to assess interspecific variability. More recently, emphasis was placed on the creation of species-specific primers and DNA probes to be used in end-point and semi-quantitative/quantitative PCR (real-time PCR). These techniques make diagnostic procedures more effective and accessible, even to scientists not specialised in taxonomy. Several protein coding genes (actin, aldolase, beta-tubulin and hsp90) were recently used for molecular characterisation in cyst nematode diagnostics.

HISTORY

Ferris *et al*. (1993, 1994) were the first to sequence the internal transcribed spacers of ribosomal RNA genes (ITS-rRNA) from several isolates of cyst nematodes belonging to the genus *Heterodera,* and to compare the sequences with those published from *Caenorhabditis elegans*. Universal primers used for PCR allowed amplification of the complete ITS1, 5.8S gene and ITS2 of the rDNA array, including parts of the 18S and 28S genes adjacent to the spacer regions. The ITS sequence data of the cyst nematodes were highly dissimilar to those of *C. elegans*. The sequences for five geographic isolates of *Heterodera glycines* were very similar to one another, but showed nearly as many differences as between this species and either *H. schachtii* or *Heterodera trifolii*. More differences were observed between *H. glycines* and *Heterodera carotae* or *Heterodera avenae.* Despite some mistakes in these newly obtained sequences, these findings confirmed the usefulness of gene and spacer regions of rRNA genes when looking for systematic inference among species and genera of cyst nematodes, and for identification as well. A few years later, Szalanski *et al*. (1997) examined the ITS1, using nucleotide sequencing and PCR-RFLP to assess intraspecific variation between and/or within European, Asian and North American isolates of five heteroderid species. The PCR-RFLP patterns of *Heterodera goettingiana* from Northern Ireland were identical to patterns from the state of Washington. However, sequencing demonstrated that ITS1 heterogeneity existed within individuals and between isolates, but did not result in different restriction patterns. Sequencing of three Indian and two American *Heterodera zeae* isolates revealed variation among ITS1 clones from the same individual, between

individuals, and between isolates. An additional, variant ITS1 region present in the isolates from the US but not in the Indian isolates, created a composite PCR-RFLP pattern. The authors concluded that heterogeneity might contribute to the complexity of the restriction digestion pattern and can serve as highly specific genetic markers.

From 1997 to 2009, a number of articles contributed to the expansion of ITS-RFLP profiles and sequences of more than 40 different species of cyst nematodes belonging to the genus *Heterodera*. The conclusion drawn from new species descriptions, identification and phylogenetic studies was that ITS-RFLP profiles and sequences are most useful for *Heterodera* species identification. Restriction of the ITS amplicons with one or a combination of seven restriction enzymes (*Alu*I, *Ava*I, *Bsh*1236I, *Bsu*RI, *Cfo*I, *Mva*I, and *Rsa*I) enables discernment of agriculturally important cyst nematode species, both from one another and from their sibling species (Subbotin *et al*. 2000). Species of *Heterodera* from the Avenae group (*H. arenaria*, *H. aucklandica*, *H. australis*, *H. avenae*, *H. hordecalis, H. filipjevi*, *H. mani*, *H. latipons*, *H. pratensis* and *H. ustinovi*) can be differentiated from one another using the enzymes *Alu*I, *Cfo*I, *Hinf*I, *Ita*I, *Pst*I, *Rsa*I, *Taq*I and *Tru*9I (Subbotin *et al.* 2003). Restriction profiles for Chinese populations of cereal cyst nematodes were published recently by Ou *et al.* (2008a). The studies also revealed that heterogeneity is present in several *Heterodera* species, resulting in composite RFLP profiles that depend on the enzymes used. Subbotin *et al*. (1999) distinguished two types of ITS regions within *H. avenae*. Bekal *et al*. (1997) also observed polymorphism between *H. avenae* populations, but those observations differed from those of Subbotin *et al*. (1999). Szalanski *et al*. (1997), Subbotin *et al*. (2000, 2003), Wouts *et al*. (2001) and Zheng *et al*. (2000) reported intraspecific variations within the *Heterodera* species *H. betae*, *H. carotae*, *H. ciceri*, *H. cruciferae*, *H. filipjevi*, *H. glycines*, *H. pratensis*, *H. schachtii, H. trifolii, H. urticae* and *H. zeae*. Madani *et al*. (2004) and Rivoal *et al*. (2003) reported a relatively high level of sequence divergence between populations of *H. hordecalis,* and suggested that two species may be grouped under this taxon. The same level of sequence divergence was observed between *H. latipons* populations (Rivoal *et al*. 2003). This is likely a case of sibling species, as Ferris *et al.* (1999) proposed earlier. The number of genetically different populations within the same species may be even higher than what has been observed so far.

As the new millennium began, another approach to *Heterodera* species identification was being developed. In 2001, Amiri *et al*. designed a primer, using the available ITS-rDNA sequence information, that is specific for species from the *H. schachtii sensu stricto* group. The primer was evaluated with 30 populations and species within this *Heterodera* group, as well as several other parasitic nematode species. Subsequent digestion of amplified PCR product by *Mva*I and *Pvu*II allowed separation of the morphologically poorly distinguishable *H. schachtii*, *H. betae* and *H. trifolii* from one another. This method of identification is highly sensitive: amplification was obtained even when a single second-stage juvenile or a single cyst was mixed with other nematode species. In the same year, Subbotin *et al*. (2001) described a method to rapidly identify juveniles and cysts of the soybean cyst nematode, based on PCR with species-specific primers (Figure 1). The PCR assay was tested on 53 populations originating from China, Russia, the US, and Brazil. This method could detect a single cyst or second-stage juvenile of *H. glycines,* alone or in a mixture with other soil-inhabiting nematodes. In 2002, Amiri *et al*. supplemented their research with a species-specific primer to detect only *H.*

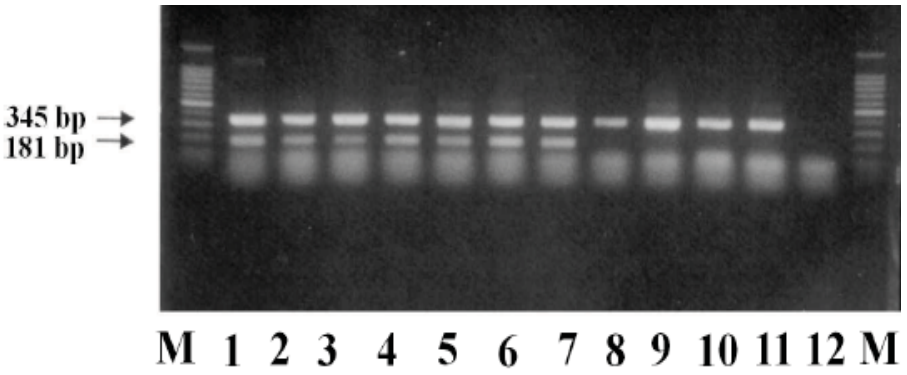

Figure 1. Duplex PCR with *H. glycines* specific primer resulting in the *H. glycines* specific 181 bp and in the universal 345 bp PCR product. M: 100 bp DNA ladder; 1-7: *H. glycines*, 8: *H. schachtii*, 9: *H. ciceri*, 10: *H. medicaginis*, 11: *H. cajani*, 12: negative control (Subbotin *et al.* 2001).

schachtii. Recently, a PCR test with species-specific SCAR primers for *H. glycines* was developed by Ou *et al.* (2008b). In 2005, Madani *et al*. (2005) used the *H. schachtii* specific primer in combination with SYBR green I dye to detect and quantify *H. schachtii* nematodes in samples using real-time PCR. Real-time PCR is faster than end-point PCR, especially since it eliminates the time-consuming post-PCR agarose gel electrophoresis.

PRESENT STATUS

Currently, most of the agriculturally important cyst-forming nematodes of the genus *Heterodera* are identified by using PCR-ITS-RFLP and sequencing of the ITS-rRNA genes, and PCR using species-specific primers developed for *H. schachtii* and *H. glycines*. However, PCR-ITS-RFLP diagnostics profiles have only been generated for 40 species, half of the valid *Heterodera* species, whereas another 40 known species have not been molecularly characterised. For several *Heterodera* species (*H. avenae, H. carotae, H. filipjevi, H. hordecalis, H. latipons* and *H. salixophila*), interspecific RFLP polymorphism with one or several restriction enzymes has been reported. This should be taken in account when diagnosing these species. Several studies have also revealed that, in some cases, identical ITS sequences can be found in morphologically clearly distinct *Heterodera* species such as *H. avenae* and *H. arenaria, H. carotae* and *H. cruciferae* (Subbotin *et al.* 2000), and *H. trifolii* and *Heterodera daverti* (S. A. Subbotin, unpublished data). Consequently, identification of these species should be based on IEF profiles of proteins and/or morphometric characteristics, until reliable markers for differentiation of these species have been published.

PROSPECTS AND RECOMMENDATIONS

It is clear from the work over the last 15 years that molecular techniques are powerful tools for nematode diagnosis. Using these tools, scientists have solved a number of problems, but an even larger number remain unsolved. The promising

and encouraging results have increased demand for better-performing techniques and applications in new fields. People now expect faster diagnostic results, preferably with on-the-spot sample examination. Direct diagnosis from soil samples, without first extracting the nematodes, would make diagnosis even faster.

Mobile molecular equipment may be particularly useful in quarantine applications, where the detection of single individuals is of paramount importance. Techniques must also be able to distinguish between dead and living individuals. Molecular nematode diagnostic techniques are evolving toward nanodiagnostics, which is still primarily in the research stage.

Recent progress in sequencing nematode genomes, including the recent sequencing of the *H. glycines* genome, supports the search for more reliable markers for use in diagnostics. DNA sequencing costs have decreased more than 100-fold over the past decade, fuelled in large part by tools, technology and process improvements developed as part of the successful effort to sequence the human genome. New technology opens the door to the next generation of sequencing methods, which include, pyrosequencing, sequencing-by-synthesis and sequencing using nanopores. There are many opportunities to reduce the cost and increase the throughput of DNA sequencing, which are likely to lead to very different and novel approaches to diagnostics (Perry *et al.* 2007).

ACKNOWLEDGMENT

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THE LIFE CYCLE OF *HETERODERA FILIPJEVI* IN WINTER WHEAT UNDER MICROPLOT CONDITIONS IN IRAN*

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SUMMARY

Cereal cyst nematode, *Heterodera filipjevi,* is the dominant species in most wheat growing areas in Iran. The life cycle of *H. filipjevi* was studied on the winter wheat cultivar Sardari in microplot under rain-fed field conditions for two years. The process of nematode development was variable during the two years due to differences in seasonal temperatures and precipitation. The penetration of second-stage juveniles (J2) was observed in early December and late November in first and second year respectively when the soil temperature was 10.0C and 10.4C. White females were visible on root in the first week of April and late March in first and second year with soil temperature 13.3C and 12.0C, respectively. Cyst formation occurred one month after observing white female in both years. The results showed that *H. filipjevi* developed only one generation per growing season and completed its life-cycle within 155 (150±10) days in wheat. The J2 of *H. filipjevi* were found in the soil from November through March under the experimental conditions. The completion of white female development and eggs containing embryos took 209 and 358 degree days, respectively, with the base temperature of 8C.

INTRODUCTION

Cereal cyst nematodes (CCN) are known to be a major constrain to wheat production in several parts of the world. Three species of CCN are documented to be the most economically important in wheat are *Heterodera avenae*, *H. filipjevi* and *H. latipons* (Nicol and Rivoal 2008).

In Iran, *H. filipjevi* is widely prevalent in most wheat growing regions of the country (Tanha Maafi *et al*. 2007). The yield loss due to this nematode can reach 42% in several rain-fed winter wheat locations in Turkey (Nicol *et al*. 2006). Furthermore, grain yield losses caused by *H. filipjevi* were calculated at 11 to 47% for an initial

*Hajihasani A, Tanha Maafi Z (2009) The life cycle of *Heterodera filipjevi* in winter wheat under microplot conditions in Iran. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 115-117. (CIMMYT: Ankara, Turkey)

population densities of 2.5 to 20 eggs and J2/g soil for winter wheat in Iran (Hajihasani *et al.* 2008). The aim of this study was to investigate some aspects of the life cycle of *H. filipjevi* in susceptible wheat cv. Sardari in a microplot under field conditions.

METHODS

The two experiments were conducted in microplot under natural field conditions during the 2007/8 and 2008/9 growing seasons. Each year in early November, seeds of wheat cv. Sardari were sown in a microplot 1.5 × 1.5 × 0.6 m filled with soil naturally infested with *H. filipjevi*. Population density of eggs and J2 were determined in the beginning of the experiments and at harvest. Meanwhile, root and soil samples were collected at 5-7 day intervals throughout the season to follow the hatching process and developmental stages. The minimum and maximum temperatures of soil were recorded throughout the experimental periods.

RESULTS

The process of nematode development was variable during the two years due to the differences in seasonal temperatures and precipitation. The penetration of J2 was observed in early December and late November in first and second year, respectively, when the soil temperature was 10.0C and 10.4C. Peaks in hatching occurred at two stages; first, after seedling emergence and increasing of root system, and; second, after snow melt in winter, just when the wheat starts to grow again. The white females were visible on the roots in the first week of April and late March in first and second year with soil temperature 13.3C and 12.0C, respectively. Cyst formation occurred one month later in both years. The results showed that *H. filipjevi* developed only one generation per growing season and completed its life-cycle within 155 (150±10) days.

DISCUSSION

This study showed that the life cycle of *H. filipjevi* is similar to other cereal cyst nematodes, *H. avenae* and *H. latipons*. *H. filipjevi* completed only one generation per growing season. Although hatching of juveniles was delayed two weeks in the first year compared to the second year, more juveniles were found in the rhizosphere during December. Observations in Turkey found mean peak hatching of *H. filipjevi* in November (11%) and March (11%) in the first growing season and in a second growing season, in November (10%), December (11%) and March (38%) (Şahin *et al*. 2005).

Under the growing conditions in our study, J2 of *H. filipjevi* occur in soil from November through March, which was similar to the observations made in Turkey (Şahin *et al*. 2008).

Eggs with embryos were observed by mid-May in the first year and late April in the second year. Low soil temperature, particularly in the temperate region is one of the factors which has been shown to prolong the rate of juvenile development in *Heterodera* species (Mulvey 1959). The degree days above 8C required by *H.*

filipjevi to develop from J2 invasion to eggs with embryos was determined at 358 (384-332) day degrees. That was greater than that required by *H. latipons* in Cyprus and Syria in barley (Philis 1999, Ismail *et al.* 2001). This suggests that *H. filipjevi* probably requires greater energy for development and completion of the life cycle.

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CEREAL CYST NEMATODES IN ISRAEL, AND THEIR BIOLOGY AND CONTROL STRATEGIES*

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SUMMARY

The susceptibility of *Triticum durum* cultivars and host status of common poaceous weeds in Israel to some domestic populations of *Heterodera avenae* and *H. latipons* were evaluated, as well as the efficacy of abamectin as seed dressing in *Triticum aestivum*. Among the tested poaceous weeds, only *Hordeum glaucum* and *Phalaris paradoxa* were good to intermediate hosts to *Heterodera avenae* and *H. latipons*. The difference in reproduction rate of *H. latipons* on *P. paradoxa* among tested populations suggests that there are two pathotypes of *H. latipons*. The durum wheat cultivars were more resistant than the tested wheat cultivar. Seed dressing with abamectin increased the root length and decreased the number of *H. avenae* females in the test tube experiment, but the control efficacy decreased when naturally infested soil was used in pot and bucket experiments. Experiments under field conditions are required to evaluate this nematode control technique.

INTRODUCTION

The cereal cyst nematode (CCN) *Heterodera avenae* is one the most important soilborne pathogens of wheat in Israel, where about 63% of the wheat fields throughout the country are thought to be infested with *H. avenae*, and yield may be reduced by more than 50% (Mor *et al.* 1992). The other CCN that occurs in Israel is *Heterodera latipons*, which was isolated for the first time in Israel about 40 years ago (Franklin 1969). This species is thought to cause much less damage to cereals than *H. avenae* (Mor *et al.* 1992, 2008). Currently, CCN are controlled in Israel by crop rotations. To maximise the effect of the crop rotation, host weeds of the CCN should be controlled in fields. However, host status of poaceous weeds in our region to the CCN is unknown.

*Oka Y, Gözel U, Speigel Y, Mor M (2009) Cereal cyst nematodes in Israel, and their biology and control strategies. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 118-123. (CIMMYT: Ankara, Turkey)

Chemical nematicides were proven to be a promising method for controlling *H. avenae* (Brown 1984). However, nematicide use declined as growers adopted resistant cultivars and other alternatives due to the cost and environmental concerns (Vanstone *et al.* 2008). Environmentally-friendly chemicals and their application methods may be supplementary used with other control methods. β-aminobutyric acid and phosphite (HPO_3^{2-}) inhibited nematode development of *H. avenae* in wheat (Oka and Cohen 2001, Oka *et al.* 2007). Treating the seeds with nematicides may protect the plants in their early growth stage and also reduces the amount of nematicide applied. In the last few years, abamectin has been used as a seed dressing against plant parasitic nematodes (Faske and Starr 2007).

In the present study, susceptibility of durum wheat (*T. durum*) cultivars and host status of common poaceous weeds in Israel to some domestic populations of *H. avenae* and *H. latipons* were evaluated. Nematode control efficacy of wheat seed dressing with abamectin was also tested.

METHODS

Host Range

Populations of *H. avenae* and *H. latipons* were collected from wheat fields at Bet-Dagan, Erez, Dorot and Magen, and Gilat, Rahat and Dorot, respectively. *Heterodera avenae*-infested soils were used for the host range determination, while second-stage juveniles (J2) of *H. latipons* were used for inoculation at 1000 (500 × 2) J2/plant. Wheat (*Triticum aestivum* cv. Bet-Hashita), barley (*Hordeum vulgare* cv. Ortolan) and poaceous weeds (*Hordeum glaucum*, *Lolium rigidum*, *Phalaris minor*, *P. brachystachis* and *P. paradoxa*) were evaluated. Adult females of *H. avenae* and adult females and males of *H. latipons* were counted 2 months after planting.

Durum Wheat Susceptibility

Wheat cv. Bet-Hashita and durum wheat cvs Cosmodur, Kromus, Svevo and WB881 were germinated, transplanted in sandy soil, and inoculated with 400 (100 × 4) J2 of *H. avenae* or *H. latipons*. Adult females and males of the nematodes in the soil and in/on roots were counted about 2 months after planting.

Abamectin Seed Dressing

Wheat cv. Yuval seeds were treated with abamectin at a dose of 0.4, 0.8 or 1.6 kg/t of seed. In the first experiment, treated and untreated wheat seeds were sown in 50-ml plastic tubes filled with dune sand, and inoculated with 250 *H. avenae* J2 per tube. Five plants out of 10 per treatment were uprooted 10 d after inoculation, and root length measured. The number of *H. avenae* adult females on the roots was counted 2 months after planting. In the second experiment, plastic pots (700 ml) were filled with *H. avenae*-infested field soil. The soil was kept moistened for 3 weeks, and then abamectin-treated and untreated wheat seeds were sown. Wheat root length was measured 2 weeks after planting. In the third experiment, buckets (10 L) were filled with the *H. avenae*-infested field soil, and the soil was kept moistened for 3 weeks. Treated and untreated wheat seeds (15 per bucket) were sown in the soil. Shoot dry weight, including grains, and number of new cysts were recorded 4 months after planting. Data were subjected to analysis of variance and the means were separated according to Tukey-Kramer HSD test.

RESULTS

Host Range

All the *H. avenae* populations reproduced at the highest rate on the wheat cultivar (Table 1). *Phalaris paradoxa* was a good host for all the populations. *Hordeum glaucum* was an intermediate to poor host depending on nematode population. Other plants were non-hosts or very poor hosts to all the *H. avenae* populations. The highest number of *H. latipons* females of all the populations were recorded on the wheat and barley cultivars, followed by *P. paradoxa* inoculated with the Gilat population. Similar results were obtained with the Dorot population (Table 2). *P. paradoxa* was a poor host for the Rahat population by comparison to the Gilat and Dorot populations. *H. glaucum* was an intermediate to poor host for Gilat and Dorot populations, whereas it was a very poor host for the Rahat population. Other plants were non-hosts or very poor hosts for all the *H. latipons* populations.

Table 1. Number of adult females of four populations of *Heterodera avenae* developed on wheat and poaceous weeds. nt = not tested.

Plant	*H. avenae* population			
	Bet-Dagan	Erez	Dorot	Magen
Triticum aestivum	170.0 a	96.4 a	46.8 a	140.4 a
Hordeum glaucum	109.0 a	37.7 b	9.0 b	23.4 b
Bromus tectorum	0.0 c	0.0 c	0.0 c	nt
Lolium rigidum	5.0 b	2.7 c	0.3 c	nt
Phalaris brachystachis	5.0 b	0.5 c	0.0 c	3.8 c
P. minor	0.0 c	0.0 c	0.6 c	0.0 c
P. paradoxa	126.1 a	67.2 ab	28.8 a	140.3 a

Table 2. Number of adult females of four populations of *Heterodera latipons* developed on wheat, barley and poaceous weeds. nt = not tested.

Plant	*H. latipons* population		
	Gilat	Rahat	Dorot
Triticum aestivum	91.4 a	110.0 a	46.8 a
Hordeum vulgare	105.8 a	nt	59.3 a
Bromus madritensis	0.0 d	0.0 b	0.0 b
Hordeum glaucum	21.4 c	3.0 b	8.3 b
Lolium rigidum	4.3 d	0.0 b	0.0 b
Phalaris brachystachis,	3.3 d	0.0 b	0.0 b
P. minor	5.1 d	0.2 b	0.7 b
P. paradoxa	63.3 b	4.1 b	38.8 a

Durum Wheat Susceptibility

The number of *H. avenae* females developing on the wheat cultivar was higher than those developing on the durum wheat cultivars (Figure 1a). The number of the nematode males developing on Svevo was lower than those of other cultivars. The number of *H. latipons* females on the wheat was higher than those developed on the durum wheat cultivars (Figure 1b). The number of *H. latipons* males were higher than females on all the wheat and durum wheat cultivars.

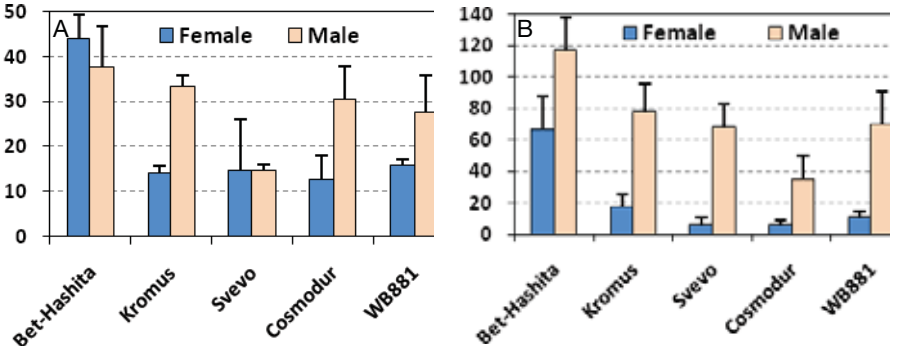

Figure 1. Number of adult females developing on *Triticum aestivum* cv. Bet-Hashita and four cultivars of *Triticum durum* inoculated with *Heterodera avenae* (A) or *H. latipons* (B).

Abamectin Seed Dressing

Roots of wheat plants emerging from abamectin-treated seeds were longer than those of the untreated seeds in both experiments (Figure 2a,b). The number of *H. avenae* adult females counted on the roots of plants emerging from abamectin-treated seeds was smaller than that on untreated plant roots (Figure 2c,d).

In the pot experiment, the number of *H. avenae* J2 per 50 g soil at seeding was 219 ± 57. Average root length of wheat plants emerging from untreated seeds grown in naturally infested soil in pots (1.6 cm) was shorter than that of abamectin-treated

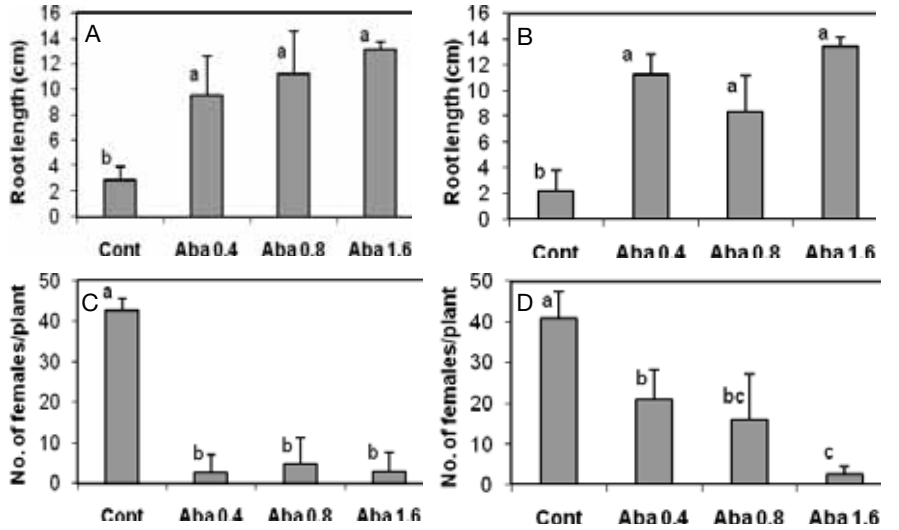

Figure 2. Root length (A,B) and number of *Heterodera avenae* females (C,D) of wheat plants grown in nematode-inoculated soil in 50-ml tubes for 10 days (A,B) and 2 months (C,D) after seeding, in the first (A,C) and second (B,D) trial. Wheat seeds were treated with abamectin (Aba) at 0.4, 0.8 or 1.6 kg/t of seed.

plants (3.5, 4.3 and 5.0 cm for seeds treated with abamectin at 0.4, 0.8, and 1.6 kg/t of seed, respectively). Increasing abamectin dose resulted in longer roots.

The number of *H. avenae* J2 per 50 g soil at seeding was 293 ± 131 in the bucket experiment. Shoot dry weight in wheat plants treated with abamectin at doses of 0.4 and 1.6 kg/t of seeds was higher than those of wheat plants from the other treatments (Figure 3a). No difference was found in the number of new cysts among the treatments, although there was a decreasing trend in the number of cysts in abamectin-treated plants (Figure 3b).

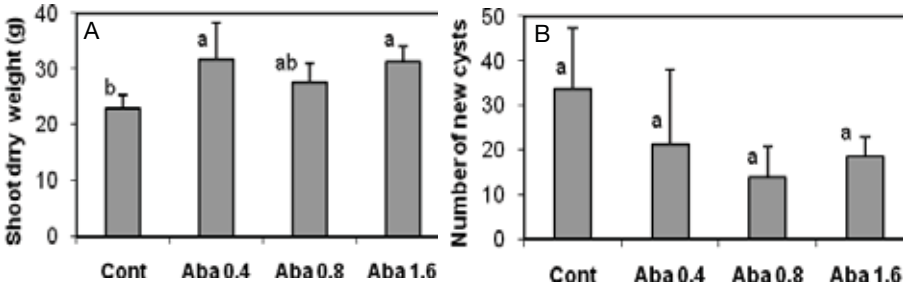

Figure 3. Shoot dry weight (A) and number of new cysts of *Heterodera avenae* per 500 g soil (B) 4 months after seeding of abamectin-treated wheat in *Heterodera avena*e-infested soil in 10-L buckets. Wheat seeds were treated with abamectin (Aba) at 0.4, 0.8 or 1.6 kg/t of seed.

DISCUSSION

Among the tested poaceous weeds, *H. glaucum* and *P. paradoxa* were good to intermediate hosts for *H. avenae* and *H. latipons*. These weeds should be controlled when crop rotation is performed to reduce populations of these nematodes. However, host status of these weeds may vary depending on nematode pathotypes. The difference in reproduction rate of *H. latipons* on *P. paradoxa* suggests that the Rahat population is a different pathotype from the Gilat and Dorot populations. Existence of *H. latipons* pathotypes has not been reported. However, the ITS rDNA sequence from an isolate of *H. latipons* from Russia was 98% similar to that of the isolate from Gilat (Ferris *et al.* 1999). Those authors suggested the possibility that the two isolates might be sibling species. Durum wheat cultivars were more resistant than the bread wheat cultivars to both *H. avenae* and *H. latipons* populations. Durum wheat may be useful in crop rotation.

In the experiment with abamectin in the tubes, root length of wheat plants from the treated seeds were dramatically longer, by as much as six fold. The treatment also reduced the number of adult females. Several reasons might explain this high control efficacy: 1) dune sand was used for the medium, which may have a lower affinity for abamectin than clay and silt; 2) the plants were inoculated only once; or 3) a very small volume of soil was used, allowing abamectin to spread throughout the medium. It is estimated that each seed was coated with between 18 to 70 μg of abamectin. Assuming that all the abamectin on the seed surface is released to the water phase of the medium, the concentration of abamectin in the water phase should be 1.7 to 7 μg/ml, which are higher than the LD_{50} for *M. incognita* (1.56 μg/

ml) (Faske and Starr 2007). In contrast with the tube experiment, naturally infested field soil (loess) was used in the pot and bucket experiments. The roots of wheat plants from abamectin-treated seeds were longer in the pot experiments than control roots. Since five seeds were sown in the pots, a considerable portion of the soil may have been covered with abamectin. In the experiment with the bucket filled with the field soil, differences in the measured parameters were small or even negligible. No difference was found in the number of new cysts among the treatments, although there was a decreasing trend on plants from abamectin-treated seeds. Only small increases were observed in shoot length and shoot dry weight in some abamectin-treated plants. The results indicate that nematode hatching and infection may have outlasted the nematicidal activity of abamectin in the field soil. Experiments under field conditions are required to evaluate this nematode control technique.

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OBSERVATIONS AND MORPHOMETRICS OF CEREAL CYST NEMATODE FROM BINZHOU, SHANDONG, CHINA*

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SUMMARY

At harvest in June 2008, serious yield loss was evident in a wheat crop in Binzhou, Shandong, China. Cysts were collected from soil samples from a wheat field. Cysts, eggs and second-stage juveniles (J2) were photographed and examined. Morphological observation of the cyst, vulval cone, eggs and J2 are described in this paper. The species was determined to be *Heterodera avenae* characterised by J2 with body length of 532±21 µm (480-577), body width of 20.3±1.2 µm (18.3-24.6), stylet length of 21.8±1.0 µm (18.4-24.0) with well-developed style knobs, dorsal oesophageal gland orifice 5.2±1.8 µm (2.4-8.6) from base of stylet, tail 64.8±4.1 µm (54.6-73.1), hyaline tail terminal 38.9±2.7 µm (33.4-45.7) and cysts were lemon-shaped, brown to dark brown, with protruding longer neck and vulva. A pair of semifenestra on vulval cone vulval underbridge absent, many bullae present obviously, vulval silt 8.8±1.5 µm (7.0-11.6).

INTRODUCTION

Heterodera spp. are significant plant parasitic nematodes of cereal crops in more than 20 countries (Nicol and Rivoal 2008). In China, *Heterodera avenae* (cereal cyst nematode, CCN) was first reported in Hubei (Chen *et al*. 1991) and causes serious economic damage, it damaged such as winter wheat, highland barley and other crops; wheat is especially damaged with reductions of 30-70% in yield. China is the largest producer of wheat in the world. In recent years, the nematode has been reported in at least 11 provinces, including Hubei (Wang 1991), Henan (Wang

*Liu J, Wu HY, Zhang GM, Yu GL, Peng DL (2009) Observations and morphometrics of cereal cyst nematode from Binzhou, Shandong, China. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 124-129. (CIMMYT: Ankara, Turkey)

1993), Shandong (Liu *et al.* 2005, Ran *et al*. 2007), Hebei, Beijing, Inner Mongolia, Shanxi, Shanxi, Qinghai, Anhui (Zheng *et al*. 1996) and Gansu (Liu 2000, Peng *et al*. 2008).

Shandong is a major producer of wheat, and in recent years CCN has been found to be widespread in many areas, including Heze (Liu *et al*. 2005), Jinnan (Ran *et al*. 2007), Liaocheng, Dezhou, Zibo, Weifang (Zhao *et al*. 2009), Binzhou and Qingdao (Li *et al*. 2002). In 2008, a wheat crop in Binzhou suffer a yield loss of about 70% because of the CCN. Soil samples were collected throughout the field to study and determine the species of CCN present.

METHODS

Cysts examined were collected from a heavily infested wheat field in Binzhou, Shandong, China. The average cyst density was 36 per 100 ml soil. Cysts, second stage juvenile (J2) and eggs were obtained from soil and associated roots. J2 were hatched from eggs in cysts kept in water at 16C following incubation at 4C for 50 days. J2 were fixed in triethanolamine formalin solution (Li *et al*. 2007) and processed to glycerine by the formalin glycerine method (Liu 1995). The cysts were collected from a 250-µm sieve by hand-picking under a stereomicroscope. Permanent slide of cyst, vulva cone, egg and J2 were made and the main morphological characters were measured under light microscopy (Liu 1995). Photomicrographs of cyst vulval cones, cysts and J2 were made with differential interference contrast microscope Nikon Eclipse 90i. All measurements are in micrometers unless otherwise stated. Ratios a, b and c were calculated according to de Man (1880).

RESULTS AND DISCUSSION

Description (Figure 1)

Cyst (n=15, in glycerine)

Body length including neck 415-677 (544: SD 74) µm; body width 278-444 (360.25: SD 45) µm. Cysts collected from soil are lemon-shaped, dark to light brown with protruding neck, vulval cone obvious, cysts ambifenestrate, underbridge inconspicuous, bullae present, vulva slit short, measuring 6.97-11.6 (8.77: SD 1.5) µm long. A pair of semifenestra on vulval cone, the vulval underbridge not obvious, bullae present, fenestra length 16.7-30.3 (21.9: SD 3.0) µm, width 10.3-29.0 (16.5: SD 2.3) µm.

Second stage juveniles (n=71)

Body vermiform, straight to slightly ventrally curved upon heat fixation. Body length 480-577 (532: SD 21) µm; body width 18.3-24.6 (20.3: SD 1.3) µm; stylet length 18.4-24.0 (21.8: SD 1.0) µm; dorsal oesophageal gland orifice (DGO) 2.4-8.6 (5.2: SD 1.8) µm from the base of the stylet knobs. Tail length 54.6-73.1 (64.8: SD 4.1) µm, length of hyaline tail 33.4-45.7 (38.8: SD 2.7) µm; the end slightly blunt; hyaline portion about 60% of the tail length. Ratios a=22.6-30.1 (26.3: SD 1.6), b=7.5-9.6 (8.2: SD 0.5) and c=6.7-8.7 (7.4: SD 0.4). Distance of median bulb from anterior end 60.4-80.5 (72.0: SD 3.4) µm. Head obviously set off with cephalic

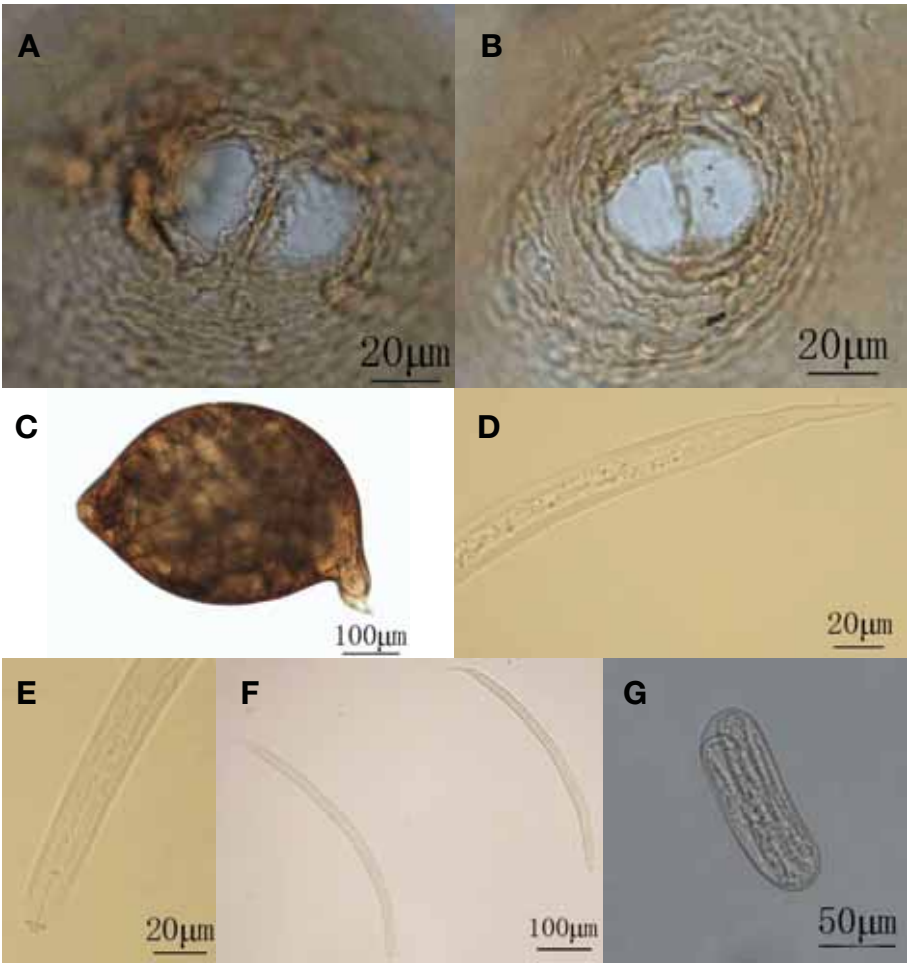

Figure 1. Photomicrographs of *Heterodera avenae* from Binzhou, Shangdong, China: A-B, ambifenestrae and vulval slit; C, whole cyst; D-E, anterior and posterior of second-stage juveniles; F, whole juveniles; G, egg.

sclerotisation. Lip region high. Stylet with prominent anchor-shaped knobs. Tail long with a conical shaped terminus.

Eggs (n=109)

Ellipsoid, length 105-141 (129: SD 6.2) μm; width 43.6-55.4 (49.6: SD 2.6) μm; length/width 2.1-3.0 (2.6: SD 0.2), colourless to light yellow; shell hyaline; juvenile folded 4 times inside egg.

Species Comparisons

According to the morphometric measurements and data in Tables 1 and 2 the Binzhou population is considered to be *Heterodera avenae*, but the cysts had shorter and narrow bodies, shorter vulval silt, and J2 with shorter bodies, stylet, tail and hyaline tail than those described from Heze (Liu *et al*. 2005) and the population described by Williams and Siddiqi (1972).

Table 1. Comparison of morphometric characters of *Heterodera avenae* from Binzhou and those described by Liu *et al*. (2005) and Williams and Siddiqi (1972).

Parameter	Binzhou (µm)	Heze, Liu *et al*. 2005 (µm)	Williams.and Siddiqi 1972 (µm)
Cyst	n=15	n=11	
Length of body	543 (415-677)	641 (420-830)	710
Width of body	360 (278-444)	428 (320-534)	500
Length of fenestrae	21.9 (16.7-30.3)	21.5 (12.0-27.5)	
Width of fenestrae	16.5 (10.3-20.0)	18.0 (10.0-23.5)	
Length of vulval silt	8.8 (7.0-11.6)	11.9 (10.5-15.0)	12-13
Egg	n=109	n=56	
Length of body	129 (105-141)	128 (114-160)	
Width of body	49.6 (43.6-55.4)	43.8 (41.0-60.0)	
Second-stage juveniles	n=71	n=39	
Length of body	532 (480-577)	582 (516-662)	510-610
Width of body	20.3 (18.3-24.6)	22.4 (20.5-29.0)	20-24
DGO	5.2 (2.4-8.6)	5.0 (3.7-6.5)	
AM	72.0 (60.4-80.5)	77.0 (66.0-87.0)	
Length of stylet	21.8 (18.4-24.0)	26.3±0.9	27 (24-28)
Length of tail	64.8 (54.6-73.1)	71.3 (37.5-87.0)	45-70
Length of hyaline tail	38.9 (33.4-45.7)	44.6 (37.5-54.0)	

Table 2. Morphological comparison of cysts of *Heterodera avenae* with other heteroderid nematodes parasitising wheat and other hosts (Mulvey 1972, Madzhidov 1981, Cook 1982, Maqbool and Shabina 1986).

Species	Shape of cyst	Type of fenestrae	Underbridge	Bullae
H. avenae	Lemon-shaped	Bifenestrae	Absent	Present
H. filipjevi	Lemon-shaped	Bifenestrae	Present	Present
H. latipons	Lemon-shaped	Bifenestrae	Present	Absent
H. hodecalis	Lemon-shaped	Bifenestrae	Present	Absent
H. bifenestra	Lemon-shaped	Bifenestrae	Absent	Absent
H. delvii	Lemon-shaped	Ambifenestrae	Present	Absent
H. zeae	Lemon-shaped	Ambifenestrae	Present	Present
H. pakistanensis	Lemon-shaped	Ambifenestrae	Absent	Absent
P. punctata	Pear-shaped	Circumfenestrae	Absent	Absent
H. iri	-	Bifenestrae	Present	Present
H. mani	-	Bifenestrae	Present	Present

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SOIL TEMPERATURE AND HATCHING OF *HETERODERA AVENAE* IN ALGERIA*

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SUMMARY

A study of the influence of soil temperature on hatching of *Heterodera avenae* over two successive years revealed emergence of 21, 37, 27, 39 and 42% of juveniles for populations of Djendel (Aïn Defla), Oued Fodda (Chlef), Dahmouni (Tiaret), Amari (Tissemsilt) and Oued Smar (Algiers), respectively. Emergence occurred during the winter period, starting in October or November and ending in April. The data indicate that temperatures from 10 to 20C are suitable for hatching, with and optimum of 14.5C for the populations studied. These populations have hatching patterns typical of the southern European ecotype of *H. avenae*, with winter emergence of juveniles and a summer-autumn diapause.

INTRODUCTION

The influence of soil temperature on the hatching of second stage juveniles (J2) of *Heterodera avenae* and their emergence cycles have been the subject of investigation under laboratory and field conditions (Meagher 1970, Rivoal 1978, 1983, Mokabli *et al.* 2001).

In Canada and in the countries of northern Europe, the emergence of juveniles starts when the temperatures reach 15 to 20C in the spring (Fushtey and Johnson 1966). By contrast, in countries where the climate is hot in summer, the hatching begins by decreasing soil temperatures in the autumn. In India, the optimal temperature for the emergence was estimated by Bhati and Malhan (cited in Zancada and Sanchez 1989) between 15 and 20C. In South Australia, Banyer and Fisher (1971) have determined that a temperature of 15C stimulated hatch.

In our investigations, we have used populations of *H. avenae* from diverse geographic origins to evaluate the effect of soil temperature on the hatching process

*Smaha D, Hamroun O, Mokabli A (2009) Soil temperature on hatching of *Heterodera avenae* in Algeria. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 130-133. (CIMMYT: Ankara, Turkey)

(release and arrest) of J2 emergence. Percentages of J2 hatching per cyst and year were calculated to evaluate the numbers of hatching cycles.

METHODS

Populations of *H. avenae* from five locations in Algeria were examined (Table 1). The study was carried out in two successive years (2005/06 and 2006/07) at the Institut National de la Protection des Végétaux experimental station.

Table 1. Location (north to south) of populations of *Heterodera avenae* sampled in Algeria, and range and mean of soil temperatures (C) for January and July for these locations.

Town (Province)	Latitude	January			July		
		Max	Min	Mean	Max	Min	Mean
Oued Smar (Alger)	36°43' N	16.2	5.6	10.9	31.2	24.9	18.7
Djendel (Aïn Defla)	36°16' N	13.6	5.3	9.4	33.5	21.4	27.4
Oued Fodda (Chlef)	36°11' N	15.5	5.9	10.7	40.2	23.7	31.9
Amari (Tissemsilt)	35°37 N	11.9	2.2	7.0	36.4	20.3	28.3
Dahmouni (Tiaret)	33°23' N	10.1	1.0	5.5	34.5	16.1	25.3

For each population, 20 cysts were individually placed in tubes with 1 ml of distilled water. Tubes were placed in a hole dug at 30 cm depth in the soil and covered with sod. The soil temperature was recorded with a thermometer placed vertically just close to the tubes containing cysts. The temperatures as daily averages were obtained, which allowed subsequent calculations of weekly averages. The J2 emergence was counted each week.

RESULTS

First emergence of J2 was from November to February for the five *H. avenae* populations in the first year and from October to January in the second year (Table 2). The commencement of the first cycle of emergence occurred at lower temperatures than the second. Hatching was finished by the end of April each year when soil temperature reached 17C.

At the end of the two hatching cycles, proportion of J2 hatch was 42, 21, 37, 39 and 27% for the populations from Oued Smar, Djendel, Oued Fodda, Amari and Dahmouni (north to south), respectively. For each population, emergence occurred systematically in the winter period with a beginning in October or November and finishing at the end of April (Figure 1).

DISCUSSION

All populations of *H. avenae* studied showed hatching cycles similar to that of other populations from Mediterranean climates. Emergence began in October or

Table 2. First emergence of juveniles of *Heterodera avenae* from cyst from five locations (north to south) in Algeria buried at 30 cm in field soil.

Year	Source location	First emergence (week - month)	J2/cyst	Soil temperature
	Oued Smar	2 - Nov	2.4	18.4
	Djendel	2 - Jan	0.4	12.3
2005/06	Oued Fodda	1 - Dec	1.9	14.8
	Amari	4 - Nov	1.1	16.5
	Dahmouni	3 - Feb	5.3	11.3
	Oued Smar	2 - Oct	0.9	20.1
	Djendel	4 - Oct	0.9	20.3
2006/07	Oued Fodda	2 - Nov	1.1	18.1
	Amari	4 - Oct	0.8	20.3
	Dahmouni	1 - Jan	3.2	11.9

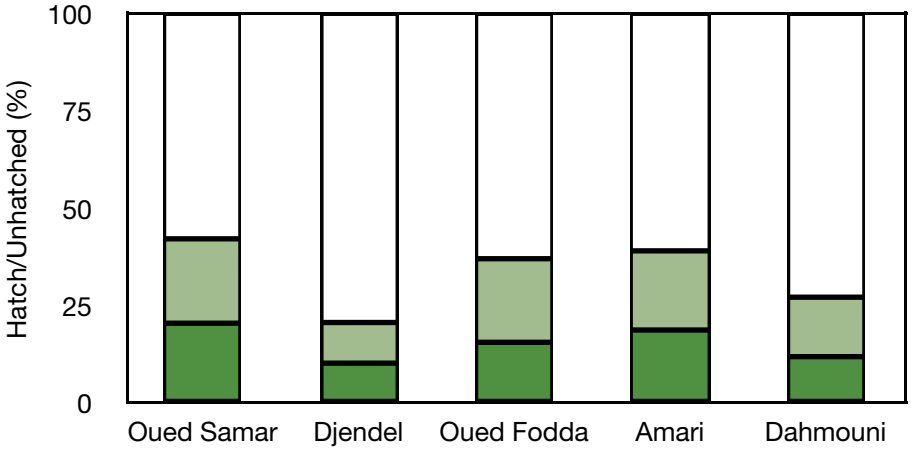

Figure 1. Proportion of hatch of five populations of *Heterodera avenae* collected in Algeria (north to south) buried at 30 cm in field soil and monitored over two successive years; 2005/06 (bottom), 2006/07 (middle) and unhatched (top).

November, when the soil temperature fell below 20.3C. The J2 cease to hatch in April when the soil temperature reached 17C again.

The Algerian populations were similar to their counterparts of southern Italy (Greco 1981, Greco and Brandonisio 1987), Spain (Romero and Valdeolivas 1990, Valdeolivas *et al*. 1991) and South Australia (Meagher 1970), where hatching occurred during the periods of lower temperatures. In Algeria, hatching was triggered by declining temperature, while increase of temperature (up to 17C) caused cessation of the hatching cycle for all populations tested.

The work confirmed that Algerian populations of *H. avenae* from the five locations belong to the Mediterranean ecotype characterised by winter hatching activity.

Furthermore, we observed that the proportion of unhatched eggs was considerable (about 60-80%) at the end of the two-year experiment, which indicates cohort of J2 which are able to hatch each year as previously noted by Meagher (1970) in Australia and Rivoal (1983) in France.

In Algeria, variation of the tolerance of cereal cultivars to *H. avenae* can be explained, in part, by the biology of the nematode. Indeed, durum and bread wheat sown in late autumn are more vulnerable to nematode attack, as their early vegetation coincides exactly with the beginning of J2 emergence. Such vulnerability of durum wheat to *H. avenae* infestations was observed also in France. In contrast, in northern Europe, spring sown cereals, such as oat or maize, are more vulnerable to this nematode because their growing period coincides with the spring activity of the northern ecotype (Rivoal and Ireholm 1990).

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PART 4 MANAGEMENT BY HOST RESISTANCE

SUCCESS OF CEREAL CYST NEMATODE RESISTANCE IN AUSTRALIA: HISTORY AND STATUS OF RESISTANCE SCREENING SYSTEMS*

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SUMMARY

Heterodera avenae resistance has been a priority for cereal breeding programs in southeastern Australian since the 1970s. Screening has been undertaken by an independent nematode screening program. Throughput was a limiting factor, so a high throughput system, able to screen over 100,000 plants annually was developed. As a consequence, now 36 of the 69 recommended cereal cultivars are resistant to *H. avenae* compared to 7 in 1987.

INTRODUCTION

Cereal cyst nematode (CCN), *Heterodera avenae*, is an important plant parasitic pest of cereals. It was first reported in Australia in 1930 (Davidson 1930) following investigation of crop damage in various districts of South Australia (SA) and western Victoria (Vic.) (Millikan 1938). By 1981, more than 2 Mha in SA and Vic. were infested with CCN and annual losses for wheat alone were estimated at A$72 mil. (Brown 1981).

In the 1930s, research in Vic. showed rotations of three years with a non-host crop or fallow reduced nematode populations (Millikan 1938), but this practice was not sufficiently profitable or practical. The possibility of using resistance to control CCN in Australia was first considered in the mid-1930s (Millikan 1938), with resistant oats shown to improve yields in following cereal crops, but resistant wheat and barley were not available at the time. Later, improved yield following resistant wheat was demonstrated experimentally (O'Brien and Fisher 1977).

In 1978, the economic impact of CCN in SA was highlighted by the use of nematicides and earlier adoption of the New South Wales rust resistant wheat c.v

*Lewis JG, Matic M, McKay AC (2009) Success of cereal cyst nematode resistance in Australia: history and status of resistance screening sytems. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 137-142. (CIMMYT: Ankara, Turkey)

Festiguay; fortuitously also resistant to CCN. Often only parts of fields were sown to Festiguay, because seed was in short supply. In the next season, vigour of cereals was dramatically improved where Festiguay had been grown (Rathjen *et al.* 1998).

Consequently CCN resistance became an important objective for southeastern Australian cereal breeding programs. To facilitate this, in 1978 the SA Rural Industry Research Funds supported a SA Department of Agriculture project to screen SA wheat breeders' lines for CCN resistance and tolerance and in 1981 a project to screen barley lines. From these projects a CCN screening service for southeastern Australia was established. The early projects primarily used two resistance testing methods: a growth room bioassay (Fisher 1982) and field testing. Breeding programs in Vic. used naturally-infested soil for an assay conducted in controlled conditions (Eastwood *et al.* 1991), but only at a limited scale.

Testing led to the release of the CCN resistant feed barley Galleon in 1981, the first commercially available CCN resistant barley bred in Australia (Sparrow and Dube 1981). More resistant cereals followed with the release of Wallaroo oat in 1988 (Barr 1988) and the first SA bred CCN resistant wheat, Molineux with Festiguay sourced resistance, in 1988 (Rathjen *et al.* 1989).

These early screening projects annually tested about 4,000 plants with the growth room Tube Test and 500 lines with the field test for SA breeders. However the grains industry saw the development of CCN resistant cultivars to be of great importance, but were concerned that the limited screening throughput was a bottleneck in the development of CCN resistant cultivars.

In 1990, the Wheat and Barley Research Committees for SA convened a meeting of breeders and researchers to develop a cost efficient system to screen large numbers of breeding lines for wheat, barley, oat and triticale From this the high throughput Pots Test was developed and implemented in 1994.

METHODS

Tube Test. The Tube Test is the long established test to select for CCN resistance, identify off-types in advanced lines and categorise the level of resistance. It has been used with some variation by various researchers. However, the method described by Fisher (1982), a modification of that used by O'Brien and Fisher (1977), is the one used in SA since the Khuzestan's, but with two differences: wheat, barley and oat are all inoculated with 100 juveniles, not 50 for oat and 75 for wheat as in Fisher (1982) and the soil used is a red Mallee sandy loam obtained from the surface of a farmer's paddock and sterilised before use.

Field Screening. Field screening using techniques similar to those of O'Brien and Fisher (1974) can test greater numbers of lines than the Tube Test and was used mainly to screen early generation material. It is no longer used, largely because of the difficulty in finding field sites heavily infested with CCN of adequate size (3-4 ha) and suitable soil type within reasonable travelling distance.

"Pots" Test. This testing system has two main elements: it has high throughput seeding and assessment systems and a purpose developed computer application, the Pots database, to manage the operations of the test. This test is conducted under

natural conditions on plant beds at the Waite Campus, Adelaide during the winter growing season of southeastern Australia, using supplementary sprinkler irrigation.

The breeders' lines are grown as single plants in black tubes filled with CCN inoculum mix (described below). The tubes are 10 cm long, 5 cm diameter at the top tapering to 4 cm at the base. Each tube contains about 160 g of inoculum mix and is supported in wire mesh crates, holding 50 tubes (5 rows by 10 columns).

The CCN inoculum mix contains CCN cysts with eggs, which hatch naturally during the test, rather than adding hatched juveniles. The mix combines a soil heavily infested with CCN and a commercial quarried sandy loam from Tailem Bend, SA that contains minimal organic material or clay. The mix is prepared in 1.4 t batches to give a CCN population density of 25 eggs/g. Osmocote Plus 8-9 Month slow release fertiliser (NPK 16:3.5:10 plus trace elements) is included in the mix at 4 g/kg together with water to produce a moist mix.

The source of the heavily infested CCN soil varies from year to year. Originally, after using heavily infested field soil, susceptible plants were retained at assessment to provide the next year's infested soil. However, recycling of the mix resulted in unacceptable build-up of *Gaeumannomyces graminis* and small-seeded weeds. Now, recycling is used only strategically and fresh field soil mostly used.

The Pots database manages breeders' information such as entry numbers and line names, generation of individual experiments with required number of replications ranging from 1 to 50 per crate, crate seeding plans, direct input into the database of assessment counts and production of electronic reports.

In June to July, crates of tubes are filled with the inoculum mix, seed sown and the crates transferred to outside beds, allowing roots to grow into the ground. A team of seven people can fill, sow and transfer about 200 crates (10,000 plants) per day.

In October to early November, when white females are visible on the roots, assessment is made by counting the females on surface of the root ball (which represents a consistently sampled proportion of the total in the root system) after removal from the pots. Assessments must be completed before the females die and turn brown. Counting is done under a magnifying lamp (2x). The assessment is non-destructive; selected plants can be repotted for seed production. Experienced operators can assess about 600 plants per day.

RESULTS

Table 1 presents an example of Tube Test results obtained for wheat and barley check cultivars. The difference between cvs Galleon and Chebec occurs consistently between tests and reflects the *Ha4* and *Ha2* CCN resistance genes, respectively. The difference between the susceptible cv. Schooner and resistant cvs Galleon and Chebec cultivars is also consistent across tests.

The difference in wheat can be less obvious, particularly as a number of resistance genes are used and give a range of reactions. Table 1 shows the reaction of wheat cultivars of known resistance and the variation that occurs in the resistant range. Cultivars Festiguay, Molineux and Frame have *Cre8,* while Chara and Annuello

Table 1. The mean, median and range of *Heterodera avenae* females produced on check cultivars in barley and wheat Tube Tests in 1998 and 2008, respectively.

Crop	Cultivar	Classification	Mean	Median	Range
Barley	Schooner	S	56.4	59	45-63
	Galleon	R	4.1	5	2–6
	Chebec	R	1.5	1.5	0-3
Wheat	Egret	S	72.0	73.0	57-83
	Spear	S	70.0	69.5	45-96
	Meering	MS	39.9	38.0	30-55
	Festiguay	MR	21.7	22.5	8-48
	Molineux	R-MR	17.3	16.0	12-27
	Frame	R-MR	11.1	10.0	5-29
	Chara	R	16.3	19.0	8-23
	Annuello	R	8.0	8.0	1-14

R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

have the *Cre1* gene. Festiguay is usually slightly higher than Molineux, but Molineux and Frame generally produce a similar result. *Cre1* gene cultivars usually have lower numbers of females than cultivars with *Cre8* in the Tube Test.

Data for check cultivars used from Pots Test is given Table 2. Low counts can occur on individual susceptible plants, but the overall result mostly indicates the correct status of the entry. However, if susceptible lines are erroneously classed as resistant, this will be revealed later testing. The erroneous classification of a resistant line as susceptible, which would cause its inappropriate elimination, is unlikely to occur.

Table 2 Examples of Pots Test counts of *Heterodera avenae* females on the root ball surface of wheat, barley and oat check cultivars.

Crop	Cultivar/line	Females/plant on root ball surface	Mean
Wheat	Aus10894	0, 1, 2, 2, 2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 4, 5, 5, 5	2.78
	Molineux R	0, 2, 2, 3, 3, 4, 4, 6, 7, 8, 9, 10, 11, 13, 14	6.4
	Egret S	0, 2, 5, 6, 8, 10, 11, 13, 16, 18, 21, 28, 30, 32, 32	15.5
Barley	Chebec R	0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 2, 3	0.6
	Galleon R	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 2, 2, 3	0.6
	Schooner S	2, 3, 6, 6, 8, 9, 10, 11, 11, 12, 14, 15, 17, 18	9.9
Oat	Wallaroo R	0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 2, 2, 3, 3	1.0
	Dalyup S	0, 1, 6, 10, 11, 11, 12, 13, 15, 17, 19, 23, 25, 36	14.2

When established in 1994, the Pots Test was designed screen 60,000 plants/year, but subsequently expanded to meet increased demand. At its peak in 2001, about 130,000 plants representing some 21,000 lines were screened. The effectiveness of screening for CCN resistance is apparent from the number of CCN resistant cultivars listed in the 2009 Cereal Variety Disease Guide with more than 50% of cultivars having CCN resistance (Table 4).

Table 3. Number of plants and lines screened for *Heterodera avenae* resistance over a 3 year period in the Pots Test.

	1999		2000		2001	
	Plants	**Lines**	**Plants**	**Lines**	**Plants**	**Lines**
Wheat	30,256	4,510	35,735	4,818	43,770	7,191
Barley	47,950	7,591	50,650	7,623	46,335	8,401
Oat	21,168	5,352	15,462	3,600	21,437	4,781
Triticale	5,100	504	5,150	503	5,000	500
Sundry	19,030	57	21,228	81	12,806	90
Total Breeders	104,474	17,957	106,997	16,544	116,542	20,873
Grand Total	123,504	18,014	128,225	16,625	129,348	20,963

Table 4. Number of cultivars resistant to moderately resistant to *Heterodera avenae* listed in the annually updated South Australian Cereal Variety Disease Guide*.

Crop	**CCN resistant cultivars**				**Total cultivars listed**			
	1987	**1991**	**2000**	**2009**	**1987**	**1991**	**2000**	**2009**
Wheat	1	2	5	10	19	23	22	28
Barley	1	1	4	12	8	8	12	17
Oat	4	7	7	10	14	18	19	17
Triticale	1	2	1	4	3	4	3	7
Total	7	12	17	36	44	53	56	69

*Wallwork H, Dube AJ *et al.* (1987, 1991, 2000 and 2009) (SA Government: Adelaide)

DISCUSSION

Commitment to screening cereal breeding lines over the past 30 years for CCN resistance using phenotyping methods and more recently molecular markers has been an outstanding success. While the Tube Test and Field Screening were significant contributors to the release of resistant cultivars, the Pots Test has had the greatest impact. The capacity to screen 21,000 lines compared to 500 lines by Field Screening has enabled the breeding of increased numbers of resistant cultivars.

The Pots Test method of counting females on the root ball surface rather than in the whole root system can misclassify some individual susceptible plants with low counts. This has not detracted from the effectiveness of the test because the low cost and high efficiency means escapes will be detected in subsequent testing.

The breeders have developed various strategies in their use of the test. This includes varying the number of replications per line depending on its breeding program stage, testing single head selections of a line for homogeneity and reselecting early generation material. Screening for resistance in wheat can be more complicated, so 20 plants per line are split into four blocks of 5 plants in different crates to minimise the risk of misclassification.

Confidence in the ability of the Pots Test to identify resistant lines was demonstrated by Safari *et al.* (2005) who analysed Pots data over a five year period and showed

that lines with the resistance genes *Cre1*, *Cre3* and *Cre8* had significantly lower counts than without a resistance gene.

The availability of 36 cultivars resistant to *H. avenae* of the 69 cultivars listed in the 2009 Cereal Variety Disease Guide attests to this capability. The present high incidence of fields with low, non-damaging levels of CCN (Riley and McKay, 2009) shows the success of the adoption and usefulness of using resistance to control CCN. However, if the frequency of susceptible cultivars planted were to increase, CCN populations will quickly return to damaging levels.

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IMPORTANCE OF CEREAL CYST NEMATODE IN RAJASTHAN, INDIA AND ITS CONTROL THROUGH BREEDING FOR RESISTANCE*

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SUMMARY

Of some 80 species of plant parasitic nematodes associated with wheat and barley only two are considered to be key pests in India, namely the cereal cyst nematode (CCN), *Heterodera avenae* and *Anguina tritici*. As genetic resistance is considered the most suitable control, breeding for resistance (particularly for wheat and barley) is in progress at Agricultural Research Station, Durgapura, Jaipur under All India Coordinated Research Project of Wheat and Barley Improvement. Thousands of genotypes of barley and wheat, both exotic and indigenous, obtained from CIMMYT and ICARDA have been tested against *H. avenae*. Only a relatively small number of exotic and indigenous lines of barley and wheat exhibiting resistance to CCN have been identified during many years of screening. Barley lines Morocco, CI3902, CI8334, Marocaine, PI253826 (exotic), C-164, PL-101, DL-69 (indigenous) and wheat AUS 15854 were found to be resistant to CCN. These genotypes were used as resistant donor in a breeding program to transfer resistance gene to agronomical superior local cultivars of barley and wheat. As a result, some resistant cultivars of barley including Rajkiran, RD2052, RD2035, RD2508 and RD2624 were developed incorporating resistant genes from exotic sources such as Morocco and Marocaine and an indigenous source, PL-101. Similarly, Raj MR-1, resistant a cultivar of wheat was developed using resistance from AUS 15854. Most of resistant cultivars developed at ARS, Durgapura, Jaipur are targeted at a single CCN species (*H. avenae*) and even a single pathotype (Ha41) and were tested against the local population. However, all these cultivars of barley and wheat were found to be susceptible to *Heterodera filipjevi*, which occurs in Punjab, Himachal Pradesh and adjoining districts of Haryana State of India.

*Bishnoi SP (2009) Importance of cereal cyst nematode in Rajasthan, India and its control through breeding resistance. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 143-148. (CIMMYT: Ankara, Turkey)

INTRODUCTION

There are about 80 species of plant parasitic nematodes associated with wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), but in India only two are considered to be key pests, namely the cereal cyst nematode (CCN), *Heterodera avenae*, the causal organism of Molya disease, and *Anguina tritici*. CCN was reported for the first time in the India from Sikar District of Rajasthan (Vasudeva 1958). Subsequently, it was found in many other locations both in and outside Rajasthan. Now, it is recognised as a serious problem in eleven states of northern India, especially in sandy areas of Rajasthan and adjoining Haryana. In India, CCN occupies significant status in Rajasthan because of lighter sandy soils and large area infested (150,000 ha) causing about US$2.5 million losses every year (Sharma and Sharma 2000). In individual fields, sometimes losses may reach 50-66% or more depending upon on the severity of the nematode infestation (Mathur *et al.* 1980).

To overcome from this problem, many control measure like summer ploughing, crop rotation, soil amendment with organic matter, application of pesticides and resistance breeding were tried, with resistant cultivars being recognised as the most promising method for managing *H. avenae*. Since the discovery of resistance in barley (Nilsson-Ehle 1920) and wheat (O'Brien and Fisher 1974), control of CCN through the incorporation of resistance into commercial cultivars is to be considered easy, economical and safe method.

Therefore, since 1958, attempts have been made to discover resistance sources both in exotic and indigenous germplasms of barley and wheat against the local population of CCN in India. After finding and confirming suitable resistance sources in barley (Bhatnagar *et al*. 1965) and wheat (Mathur *et al*. 1980) for the local CCN population, attempts were made to transfer the resistance gene (*Rha3*) from barley and wheat to commercial cultivars by simple hybridisation methods. A resistance breeding program, focusing on wheat and barley, is based at the Agricultural Research Station, Durgapura, Jaipur under All India Coordinated Research Project of Wheat and Barley Improvement. In this report, summarises the efforts and achievements of this program in finding resistance sources and development of CCN resistant cultivars.

METHODS

Thousands of genotypes obtained from exotic (such as CIMMYT, ICARDA and Australia) and indigenous sources (National Bureau of Plant Genetic Resource, New Delhi and Directorate of Wheat Research, Karnal) were screened every year as 25 replicates in fields heavily infested. Resistant lines obtained from the field screening were further tested for CCN reaction in pots by inoculating with CCN second stage juveniles. Two seeds of each genotype were sown in l0-cm-diameter earthen pots containing 500 g sterilised alluvial soil sand mixture (1:1) and thinned to one plant per pot after germination. Pots were irrigated regularly with distilled water to avoid any contamination. Ten-day old plants were inoculated with 4 J2/ml soil (2,000 J2/ pot). Two sets of pots were maintained (with ten pots of each genotype), one for uprooting at 75 days and the other at 90 days after inoculation. The roots of plants uprooted at 75 days after inoculation were washed with tap water and stained in acid fuchsin to facilitate counting the juvenile stages in the roots under a stereomicroscope (Byrd *et al.* 1983). The plants uprooted at 90 days after

inoculation were used for counting the number of white females per plant. After confirmation of resistance, both in field as well as in pots, the resistant genotypes were used in breeding programs to transfer resistance gene to commercial cultivars. Most of genotypes identified as CCN resistant through screening were not agronomical suitable for the area. To obtain the desired agronomical traits along with CCN resistance, parents were selected according to local requirements. Morocco, Marocaine and PI253826 from exotic and PL101 from indigenous barley were used as CCN resistant donor parents. For wheat AUS15854 was used as donor parent. Simple hybridisation pedigree and back crossing methods were used. The F1 seeds were sown separately to get the F2 generation. All crosses were sown in microplots of 1 m^2 each consisting of three rows of 8-10 plants. After segregation of genotypes, those found to be resistant to CCN were selected and further tested in the same way. Based on the number of females developing, the plants were categorised as resistant (0-4 females/plant), moderately resistant (5-9 females/plant) and susceptible (10 and above females/plant) according to Bishnoi (2000). Resistant lines were bulked and after yield evaluation released for cultivation in CCN infested areas.

RESULTS

Wheat

Three resistant (Raj MR1, CCNRV2 and CCNRV4), and one susceptible check (Raj 1482) wheat cultivars developed by our program were studied for the development of CCN and yield potential. The number of J2 penetrating the roots of resistant wheat cultivars was in the range 12.8-15.3/plant compared with the 39.8 in the susceptible check (Table 1). The penetration of the nematode into resistant cultivars was significantly less and they were unable to develop into mature females in the three resistant cultivars. The number of females developing per plant was almost negligible (0 to 2) in resistant cultivars. Our results agree with findings of Pankaj *et al.* (2006). The yield was also significantly higher in the resistant cultivars compared to the susceptible check. One of the resistant cultivars (Raj MR1) has already been released for commercial cultivation in *H. avenae* infested areas of Rajasthan in India.

Barley

Seven barley genotypes were studied the reaction and development of CCN (Table 1). The penetration of the nematode into a resistant cultivars was significantly less. The number of white females developing in resistant cultivars were significantly less, with four cultivars categorised as resistant (Raj kiran, RD2052, RD2035 and RD2508) and two (RD2624 and RD2660) as moderately resistant. These seven cultivars have been released for commercial production.

DISCUSSION

In this study, seven cultivars of barley and four cultivars of wheat developed at Durgapura were tested against Jaipur CCN population. Rajkiran (RD387), a cross between RDB1 and Marocaine (with *Rha3* gene) was found resistant against Jaipur population but susceptible to Ambala, Punjab and Himachal Pradesh populations (Bishnoi 2000). Bhatti and Jain (1993) have also mentioned that Rajkiran was

Table 1. Reaction and yield potential of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) cultivars developed with resistance to cereal cyst nematode (*Heterodera avenae*) in India.

Cultivar	Parents	Resistant source	J2/plant	Females/ plant	Reaction	Yield (t/ha)	Status
Wheat							
Raj MR-1	J24 x AUS15854	AUS15854	12.8	1.3	R	3.88	Released
CCN RV_2	Raj2184 x AUS15854	AUS15854	15.3	1.5	R	3.43	Not Released
CCN RV4	Raj2329 x AUS15854	AUS15854	13.5	2.0	R	3.25	Not Released
Raj1482	Susceptible check	-	39.8	27.3	S	1.95	Released
Barley							
Raj kiran	RDB 1 x Marocaine	Marocaine	16.3	0.0	R	3.39	Released
RD2052	(ApiCM67 x So727) POR XPL101	PL101	24.0	2.5	R	4.22	Released
RD2035	RD137 x PL101	PL101	19.7	2.8	R	3.94	Released
RD2508	RD2035 x PL490	RD2035	32.3	4.5	R	2.46	Released
RD2624	BL2 x RD2508	RD2508	36.0	8.3	MR	2.40	Released
RD2660	RD2052 x RD2566	RD2052	38.7	9.8	MR	2.07	Released
RD103	Susceptible check	-	56.7	39.5	S	1.95	Released

susceptible to Ambala population. In this breeding program, cultivar Marocaine has been used as resistant donor, being resistant to most of the CCN populations of world but susceptible for 'British pathotype 3' and Glieneitz populations of West Germany. These populations of Britain and West Germany are now considered to represent *Heterodera filipjevi.* Ambala, Punjab and Himachal Pradesh population of India is now known as *H. filipjevi.* Due to transfer of undesirable genes from Marocaine responsible for delayed maturity in Rajkiran, it was desirable to find an indigenous source of resistance. Subsequently, some resistant sources like DL69 and PL101 were identified. Resistant gene from PL101 was subsequently transferred into a local commercial background and resistant cultivars RD2035 and RD2052 were released. However, these two cultivars have not been tested extensively against different populations of India. Cultivar RD2035 was found to be resistant to the Jaipur population but susceptible to Ambala, Punjab and HP population. The differences in the reaction may be attributed to the existence of more than one pathotype in India (Mathur *et al*. 1974). The different patterns of reaction are most probably due to presence of different species *(H. filipjevi* and *H. avenae sensu stricto*) and pathotypes (Hf31, Hf41 and Ha21). Breeding for resistance using standard techniques is time and resources consuming, so it is imperative that the species and pathotypes are well characterised.

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CURRENT GLOBAL KNOWLEDGE OF THE USABILITY OF CEREAL CYST NEMATODE RESISTANT BREAD WHEAT GERMPLASM THROUGH INTERNATIONAL GERMPLASM EXCHANGE AND EVALUATION*

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SUMMARY

One of the most cost effective, environmentally friendly and easily adopted control measures is the use of genetic host resistance which will maintain nematode populations below economic threshold for damage. Globally many sources of resistance have been reported against cereal cyst nematodes (CCN), however their effectiveness and usability is dependent on the reaction of the specific species and pathotype which are found in different regions. As part of a joint International CIMMYT-Turkey collaboration 57 entries and International Root Disease Resistance Nursery were distributed globally to access their effectiveness of the resistance for a number of soil borne pathogens of wheat including cereal nematodes and dryland root rots. Twelve of these entries are discussed in this paper. Data for

*Nicol JM, Ogbonnaya F, Singh AK, Bishnoi SP, Kanwar RS, Li HL, Chen SL, Peng DL, Bolat N, Şahin E, Elekçioğlu İH (2009) Current global knowledge of the usability of cereal cyst nematode resistant bread wheat germplasm through international germplasm exchange and evaluation. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 149-153. (CIMMYT: Ankara, Turkey)

CCN was compiled from Turkey, Australia, three locations in China and in India based on replicate data produced under *in vitro* conditions against local populations of both *Heterodera filipjevi* and *H. avenae*. Results indicated differential response against CCN pathotype Ha13 in Australia. There was also marked differences in reaction against populations of *H. avenae* from Hebei and Henan in China, and the population from Hisar, Karnal and Rajasthan, India. Some of the wheat gentoypes appear to provide effective resistance in different location. Based on these data, a new host differential set with only bread wheat sources has recently been assembled. These have been distributed to more than ten partner countries to assist in the deployment of effective resistance against soil borne pathogens for international wheat improvement.

INTRODUCTION

The cereal cyst nematode (CCN) is globally acknowledged to be an economically important biotic constraint in predominately rain-fed wheat production systems in many wheat growing regions including Australia, USA, China and India and several countries in west Asia and north Africa (Nicol and Rivoal 2008). In Turkey the predominant species on the rain-fed winter wheat productions system of the Central Anatolian Plateau is *Heterodera filipjevi* (Şahin *et al*. 2009). In China and India a number of different populations of *Heterodera avenae* have been identified and India *H. filipjevi* is also reported (Peng *et al.* 2009, Singh *et al.* 2009). Australia fortunately has only one species and pathotype of CCN, *H. avenae* pathotype Ha13.

As reviewed by Nicol and Rivoal (2008), although integrated pest management options are well researched for CCN, especially rotation with non-hosts, the use of genetic host resistance is considered on the more cost effective, environmentally friendly, accessible and effective methods of maintaining CCN populations below economic thresholds of damage. This is in part due to the single dominant gene relationship which has been established for many of the sources of resistance such that the resistance is highly effective and relatively easy to breed with. However, CCN is highly complex nematode with various species and pathotypes resulting in range of differential reactions. Thus, we designed a study to clarify the relationship between the designated sources of resistance in bread wheat and their specificity against *H. avenae* and *H. filipjevi*. The information generated will assist in choosing resistance sources for crossing in breeding and pyramiding to generate wheat germplasm in locally adapted genetic background with desirable levels of resistance to soil borne diseases.

METHODS

The most effective method to screen for resistance is under controlled greenhouse conditions with mass culture of CCN. Adopting such published methods this work has been successfully initiated in Turkey over the past five years (Nicol *et al.* 2007). As reviewed by Nicol and Rivoal (2008) resistance against the closely related species *H. avenae* has been widely published with six out of the seven published *Cre* resistance genes coming from wild relatives of wheat (*Aegilops* spp.).

Fifty seven wheat lines were identified globally with resistance to one or more of these pathogens through the joint CIMMYT-Turkey efforts to work on soil borne

pathogens with other international partners. These were compiled and distributed by CIMMYT to more than 24 partners globally, with 14 of these in Australia and 10 overseas (Nicol *et al.* 2008). The material was screened under replicated greenhouse or field conditions against local pathogens of importance. For CCN colleagues in Australia, Turkey, China and India participated in screening the nursery for their CCN reaction, under both controlled *in vitro* greenhouse conditions (Turkey, Australia, and Hebei China), while in India pots with field naturally infested field soil were used and in Henan China, plants were assessed under natural field conditions. At least three replicates of each line were used, and in most cases seven. All data was statistically analysed with ANOVA, and resistance categories were then defined as followed based on the number of CCN females per individual root system where R = resistant (<5 females), MR = moderately resistant (5-10 females), MS = moderately susceptible (10-14 females), S = susceptible (15-25 females) and HS = highly susceptible (>25 females).

RESULTS

Results are presented in Table 1 and clearly demonstrate that a range of virulence exists between the populations from different countries, regions and within countries. There were marked differences in *H. avenae* populations from different countries and regions within countries. Both *CreR* and *Cre1* were effective sources of resistance against *H. avenae* pathotypes Ha13 in Australia and the local populations of *H. avenae* in Henan, China and *H. filipjevi* in Turkey. The *Cre3* and *Cre8* genes were also effective in Australia. Additionally, *Cre3* also proved to be moderately effective against the populations of *H. avenae* in Hebei, China, but not Henan. In China, the Australia line, VL411R that is susceptible to Ha13 appears the most useful in both Henan and Hebei. Interestingly, none of the designated sources of CCN resistance in this study appeared to provide effective resistance against the populations of CCN in the three Indian provinces. However, several synthetic derivatives from CIMMYT Mexico (CROC_1/AE.SQUARROSA (224)//OPATA) which were identified for other soil borne pathogens (Nicol *et al.* 2008) including crown rot (*Fusarium* spp.) and root lesion nematode (*Pratylenchus thronei*), also appear useful for CCN resistance. Some of these are also useful in Turkey and Australia, and probably represent new sources of resistance.

DISCUSSION

As reviewed by many authors working on CCN, one of the major obstacles and challenges to using genetic host resistance is the understanding of the CCN species and pathotypes in different regions of the world where the nematode is considered to be economically important. This limited study clearly indicates that the known published *Cre* genes found in bread wheat backgrounds have a range of reaction in the regions where they were tested, both regionally and within region in some cases. This work demonstrates the importance of collecting representative populations of CCN and sharing known resistant germplasm to determine the effectiveness of such resistance in other countries with differential pathotype virulence and deployment of resistant sources to control CCN.

CIMMYT in collaboration with the Turkish Ministry and Çukurova University have recently established a controlled quarantine facility which will assist in the screening

Table 1. Reaction of selected entries from the First Root Disease Resistance Nursery to their resistance reaction of various species and populations of cereal cyst nematode (*Heterodera*) from Australia, China, India and Turkey. Results from different regional assessments. R, resistant; MR, moderately resistance; MS, moderately susceptible; S, susceptible; HS, highly susceptible (see text for criteria).

Entry[1]	Name	Gene[2]	Source[3]	*H. avenae* Ha 13 (Australia)	*H. filipjevi* (Turkey)	*H. avenae* (Hebei, China)	*H. avenae* (Henan, China)	*H. avenae* (Rajastan, India)	*H. avenae* (Hisar, India)	*H. avenae* (Karnal, India)
1	6R(6D)	*CreR*	AU	R	MR	HS	R	S	HS	S
3	CHARA	*Cre1*	AU	MR	MR	HS	MR	S	HS	S
9	SILVERSTAR	*Cre1*	AU	MR	MR	HS	R	S	HS	S
4	ID-2150	*Cre2*	SP		MS	HS	R		HS	S
10	VP1620	*Cre3*	AU	R	S	MR		S	HS	S
5	T-2003	*Cre7*	SP	S	MS		MR	HS	HS	S
7	VP5053	*Cre8*	AU	R	S	HS	S	S	HS	S
8	VL411R		AU	HS	S	R	MS	HS	HS	HS
22	CPI133859		AWCC	HS	MS			HS	MR	MR
32	CROC_1/ AE.SQUARROSA (205)//KAUZ		MX	HS	R			S	HS	MR
28	CROC_1/ AE.SQUARROSA (224)//OPATA		MX		MS			R	MR	MR
31	CROC_1/ AE.SQUARROSA (224)//OPATA		MX	R	S			MR	MR	S

[1]Entry number in First Root Disease Resistance Nursery, refer to Nicol *et al*., 2008 for more detail. [2]*Cre* gene is reported against CCN species, refer to Nicol and Rivoal (2008) for further information. [3]AU, Australia; SP, Spain; AWCC, Australian Winter Cereals Collection; MX, Mexico.

of promising sources against key representative populations from global locations where CCN is considered important. In addition most recently in collaboration with effective germplasm exchange, CIMMYT and Turkey this year distributed a revised host differential of bread wheat sources with the known *Cre* genes and new resistant materials identified in Turkey and other countries globally. Through the sharing and collating of this data the deployment of effective resistance can be better achieved. Furthermore, with CCN collections such as these potential exists to explore the phylogenic evolution of species and the related resistance differentiation which has been observed.

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MANAGEMENT STRATEGIES FOR CEREAL CYST NEMATODES *HETERODERA* SPP. IN NORWAY*

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SUMMARY

Cereal cyst nematode (CCN) populations in Norway were studied by morphological, biochemical, bioassay, and molecular techniques. *Heterodera avenae* was found to be pathotypes Ha11 and Ha12, and *H. avenae* Våxtorp. *Heterodera filipjevi* was found to be pathotype West. *H. avenae* Ha11 and *H. filipjevi* West were the most common. Twenty six barley, 21 oat and 6 summer wheat cultivars (grown commercially in Norway) were tested for their resistance to *H. avenae* Ha11 and Våxtorp, and *H. filipjevi* West. Resistance to Ha11 was found in five barley, three oat and one wheat cultivar. Resistance to *H. avenae* Våxtorp was not found in barley, but four oat and one wheat cultivars were resistant. Resistance to *H. filipjevi* West was not detected in wheat, but was in six barley and 13 oat cultivars. A management system based on careful nematode identification and knowledge of cultivar resistance was implemented in the county of Vestfold, resulting in farmers' yields improving by an average of 1 t/ha. Adopting this system throughout Vestfold could result in a gain of €800,000/year. With the increasing impact of CCN worldwide, it is concluded that correct species and pathotype identification will be essential for successful control.

INTRODUCTION

Among the cereal cyst nematodes (CCN), *Heterodera avenae* (oat cyst nematode) and *Heterodera filipjevi* (rye cyst nematode) are both of economic importance in Scandinavia (Andersson 1973, Videgård 1973, Ireholm 1990, 1994, Holgado *et al*.

*Holgado R, Andersson S, Rowe J, Clark I, Magnusson C (2009) Management strategies for cereal cyst nematodes *Heterodera* spp. in Norway. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 154-159. (CIMMYT: Ankara, Turkey)

2004). In Norway, surveys of cereal fields (Holgado *et al*. 1999, Holgado *et al*. 2003) revealed a wide distribution of *Heterodera* spp. In Norway, the losses caused by CCN can exceed 50% in infested crops and probably 1-5% nationally (Holgado *et al*. 2003), a value of €3-15 million/year. The objective of this paper is to present the basis and implementation of a system for successful management of CCN.

METHODS

CCN populations studied were 13 field populations from Norway and six reference populations (Ask, Etelhem, Norra Härene, Ringsåsen and Våxtorp) from Sweden. Ask is *H. avenae sensu stricto* pathotype Ha11. Etelhem and Norra Härene are *H. filipjevi* pathotypes East and West, respectively. Ringåsen and Våxtorp are pathotypes within *H. avenae sensu lato*. Cysts were extracted from soil samples by standard methods. Morphology of vulval cones and second stage juveniles were studied by interference contrast microscopy. The total protein profile (n = 3) of each population was studied using isoelectric focusing (IEF) and silver staining in the Pharmacia Phast II System, and analysed according to Holgado *et al.* (2004). Pathotype tests were performed as described by Holgado *et al.* (2004). Cultivars were selected from the international test assortment of Andersen and Andersen (1982). The barley differentials (with resistance genes in parentheses) were Varde (*Rha*), Emir (*Rha 'E'*), Ortolan (*Rha1*), KVL 191 (*Rha 2*) and Morocco C.I. 3902 (*Rha3*) (Andersen and Andersen 1970, 1982). The oat differentials were Nidar, Hedvig and Selma. Seed was supplied by the Nordic Gene Bank (Sweden) and Graminor AS (Norway). For molecular identification single cysts were prepared for PCR-RFLP analysis of the ITS region of ribosomal DNA. The total genomic DNA was isolated with DNeasy® kit (Hilden, Germany). The PCR product was digested with the restriction enzymes AluI, CfoI, HinfI, ItaI, PstI, RsaI, TaqI and Tru9I. Restriction fragment lengths were compared by sequence alignment with *H. avenae* complex species stored in GenBank. Phylogenetic analysis of the ITS alignment was made with TreeFinder. Twenty six barley, 21 oat and 6 summer wheat cultivars were tested for resistance to *H. avenae* Ha11 and *H. avenae* Våxtorp, and *H. filipjevi* West. The barley cv. Varde and oat cv. Nidar were included as susceptible controls. Cultivars with less than 5% newly formed females compared to the susceptible controls were considered resistant (Lücke 1976).

RESULTS

Of the 13 Norwegian populations compared morphologically to the Swedish standards, seven were identified as *H. avenae sensu stricto*, one as *H. avenae* Våxtorp, two as *H. filipjevi* and two as *Heterodera pratensis*. The population from Brekstad differed from all these species.

Eleven Norwegian populations were studied by IEF and compared to the Swedish standards. Among the 42 major protein bands some were species specific. From the banding patterns, 6 Norwegian populations were identified as *H. avenae sensu stricto*, one as *H. avenae* Våxtorp and two as *H. filipjevi*. One Swedish and one Norwegian population were classified as *H. avenae* Våxtorp. The Brekstad population differed in its banding pattern from *H. avenae sensu stricto, H. avenae* Våxtorp, *H. avenae* Ringsåsen and *H. filipjevi* populations.

Nine Norwegian populations were included in the pathotype test together with Swedish standards. According to their virulence on barley differentials, three Norwegian populations were classified as Ha11. Two populations previously characterised as *H. avenae* Våxtorp and *H. avenae sensu stricto* came out as Ha12. Four populations were close to *H. filipjevi* West, but two of them (including the Brekstad population) differed from this pathotype by failing to reproduce on oat.

In the molecular study, *H. avenae* Ha11 and Ha12, *H. avenae* Våxtorp, *H. avenae* Ringsåsen and the Brekstad population all came close to *H. avenae*. Also, a population identified as *H. filipjevi* West and another as *H. avenae* Ha.12 were both close to *H. pratensis*.

Results of resistance tests performed with Swedish standards *H. avenae* Ha11, *H. avenae* Våxtorp and *H. filipjevi* West are presented in Table 1. These species and pathotypes are common in Norwegian fields. Nine cereal cultivars (5 barley, 3 oat and 1 wheat) were resistant to *H. avenae* Ha11. Four cultivars (3 oat and 1 wheat) were resistant to *H. avenae* Våxtorp. Eighteen cultivars (6 barley and 12 oat) were resistant to *H. filipjevi* West. Resistance against *H. avenae* Våxtorp was not present in barley cultivars, but occurred in three oat and one wheat cultivar. For *H. filipjevi* West resistance was not detected in wheat. Moderate resistance against *H. avenae* Ha11 was detected in three barley and four oat cultivars. Moderate resistance to *H. avenae* Våxtorp was found in only four oat cultivars. Three barley and one oat cultivar were moderately resistant to *H. filipjevi* West.

In 2003-2004 farmers and agricultural advisers in the county of Vestfold collected post-harvest soil samples from fields with poor growth. The samples were analysed at Bioforsk. Identification by morphology was often combined with IEF to separate *H. avenae* Våxtorp from *H. avenae sensu stricto* (these species are often mixed with *H. filipjevi*). The number of viable eggs and juveniles were estimated in cyst and soil (*H. filipjevi* hatches rapidly at room temperature). Information on nematode identity and abundance were used for selecting cultivars to mitigate nematode damage to the next cereal crop. Resistant barley was generally recommended when nematode populations were high, due to its high tolerance compared to resistant oat. After 3-4 years of implementation of this system farmers have reported yield increases averaging 1 t/ha, equivalent to €800,000/year, if adopted over the whole county.

DISCUSSION

Few studies have combined morphology, protein variability (IEF), bioassays and DNA sequencing in the identification of CCN. Our studies demonstrated considerable, unexpected diversity among CCN in Norway. The data indicates the boundaries between species and pathotypes may lack clarity.

The exact identity of species and the virulence pattern both at the species and the subspecies level need further study. The methods used show some inconsistencies in species identification for certain populations. Several populations of *H. filipjevi* West did not reproduce in oat differentials. In this case, both morphology and protein patterns indicated that these populations belong to *H. pratensis*. The Brekstad population differed from all other populations in morphology and protein patterns, and was close to *Heterodera mani*. This population was collected from barley and in the pathotype tests it was virulent on several barley cultivars. Mathews

Table 1. Resistance of barley, oat and wheat cultivars to oat cyst nematode (*Heterodera avenae* pathotype Ha11 and *H. avenae* Våxtorp) and the rye cyst nematode (*Heterodera filipjevi* pathotype West). Resistance is ranked according to number of females developing in the test entry relative to the number in susceptible controls, barley cv. Varde and oat cv. Nidar.

Resistance*	*H. avenae* Ha11	*H. avenae* Våxtorp	*H. filipjevi* West
		Barley	
R	Frisco, Helium, Meltan, Otira, Simba		Antaria, Frisco, Gunilla, Iver, Pernilla, Sunnita
MR	Edel, Filippa, Iver	Arve, Fager, Gunilla, Iver, Kinnan, Vilde, Otira, Pernilla, Sunnita, Thule, Tyra	Baronesse, Filippa, Simba
MS	Fager, Gunilla, Kinnan, Vilde, Sunnita, Thule, Tyra, Ven	Baronesse, Edel, Gaute, Lavrans, Olsok, Tiril, Ven	Edel, Helium, Meltan, Tyra
S	Annabell, Antaria, Arve, Baronesse, Gaute, Lavrans, Lise, Maskin, Olsok, Pernilla, Tiril	Annabell, Antaria, Filippa, Frisco, Helium, Lise, Maskin, Meltan, Simba	Annabell, Arve, Fager, Gaute, Kinnan, Lavrans, Lise, Maskin, Vilde, Olsok, Otira, Thule, Tiril, Ven
		Oat	
R	Gunhild, Sanna, Vital	Gunhild, Sanna, Vital	Bessin, Bikini, Gere, Gunhild, Ingeborg, Kerstin, Liberto, Matilda, Olram, Pol, Sanna, Vital
MR	Hurdal, Liberto, Matilda, Svea	Biri, Hurdal, Matilda, Munin	Svea
MS	Bessin, Bikini, Biri, Gere, Ingeborg, Kerstin, Lena, Moholt, Munin	Bessin, Gere, Ingeborg, Kerstin, Lena, Liberto, Mustang, Roope, Svea	Hurdal, Lena, Mustang, Roope
S	Belinda, Mustang, Olram, Pol, Roope	Belinda, Bikini, Moholt, Olram, Pol	Belinda, Biri, Moholt, Munin
		Wheat	
R	Avans	Avans	
MR			
MS		Bajass, Bastian	
S	Bajass, Bastian, Bjarne, Runar, Zebra	Bjarne, Runar, Zebra	Avans, Bajass, Bastian, Bjarne, Runar, Zebra

*Resistance ranking: R, resistant (0-5% of females developed relative to the respective susceptible control); MR, moderately resistant (6-20%); MS, moderately susceptible (21-50%); and S, susceptible (>51%).

(1971) reported that *H. mani* did not reproduce on cereals, while another report (Cook 1982) indicates that some barley cultivars can serve as hosts. Surprisingly, the ITS studies indicated the Brekstad population to be close to *H. avenae*. This population is in many ways clearly different from *H. avenae*, and this result may indicate a need to revise the present gene library of *H. avenae*. It also demonstrates the need for using several techniques in species identification.

The morphological analysis, the protein variability and the pathotype test were based on many nematode individuals, while the ITS studies were performed using single cysts. This fact may explain some of the differences in the results. In our material the least degree of divergence in nematode identification was between morphology and IEF, so at present management systems relying on nematode identification by morphology and IEF supported by pathotype test, would offer a higher precision than offered by ITS analysis alone.

One of the most efficient methods of controlling CCN is grass-free crop rotations using non-host crops. However, this is not possible for farmers in Norway, as they have insufficient land to allow rotations free of grasses. The use of resistant cereal cultivars is considered the best method for CCN control. To be effective and durable, knowledge of species and pathotypes present in the field is essential. This is highlighted by the situation in Norway where *H. avenae* Ha11, Ha12 and Våxtorp often occur together with *H. filipjevi* West in the same field.

Our studies reveal a high variability in cultivar resistance. The range of cultivars grown changes over time, so there is a continuing need to map the virulence of species and pathotypes in the *H. avenae*-complex. Also, the dynamics of mixed populations needs stronger emphasis in CCN management systems (Holgado *et al*. 2006, Holgado and Magnusson 2007).

For *H. avenae* Ha11, the results from the pathotype tests were consistent with information provided by the breeders. Although, no intentional breeding for resistance for *H. filipjevi* West has been attempted, several cultivars were found to be resistant. No resistance to *H. avenae* Våxtorp was found in barley, but several cultivars still could have a practical value for farmers even without a full resistance (Holgado *et al*. 2007). Our results and experience show that several commercial cultivars available in Norway can be used successfully on land infested with the most common types of CCN (Holgado *et al*. 2006, 2007).

In view of the increasing damage from CCN worldwide, the recognition of the morphological and genetic variability in CCN is important for the correct identification of species and pathotypes, and consequently essential for successful management of field infestations.

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IDENTIFICATION OF GENETIC RESISTANCE TO CEREAL CYST NEMATODE (*HETERODERA FILIPJEVI*) FOR INTERNATIONAL BREAD WHEAT IMPROVEMENT*

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SUMMARY

One of the most cost effective, environmentally friendly and easily adopted control measures is the use of genetic host resistance which will maintain nematode populations below economic threshold for damage. As part of a joint International CIMMYT-Turkey collaboration more than 1000 wheat genotypes from both National and International spring and winter wheat sources have been screened under controlled *in vitro* conditions against the most commonly identified and widespread CCN species *H. filipjevi* found in the main rain-fed winter wheat production region of the 5 Mha of the Central Anatolian Plateau. To date twenty seven sources have been reconfirmed to provide useful levels of genetic resistance, with many of these being found in high yielding adapted materials. Further work is underway to validate their effectiveness under natural field conditions, in addition to these sources being shared with more than 15 countries including the several countries of west Asia, north Africa, Arab States and China, India and USA.

*Nicol JM, Bolat N, Yıldırım AF, Yorgancılar A, Kılınç AT, Elekçioğlu Hİ, Şahin E, Erginbaş-Orakcı G, Braun HJ (2009) Identification of genetic resistance to cereal cyst nematode (*Heterodera filipjevi*) for international bread wheat improvement. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 160-165. (CIMMYT: Ankara, Turkey)

INTRODUCTION

The cereal cyst nematode (CCN) is globally acknowledged to be an economically important biotic constraint in predominately rain-fed wheat production systems in many wheat growing regions including Australia, USA, China and India and several countries in west Asia and north Africa (Nicol and Rivoal 2008). Turkey is one of the ten largest wheat producers in the world with gross production of 21 Mt and average yield of 2.3 t/ha (Anon. 2009). Thirty five per cent of the total 9 Mha is under rain-fed winter wheat production on the Central Anatolian Plateau (CAP) in Turkey. Recent surveys have confirmed the widespread distribution of CCN being found in more than 78% of soil samples on the CAP wheat production system of predominately fallow wheat rotation (Şahin *et al.* 2009). The most important CCN species on the CAP is *H. filipjevi* and populations recorded in survey indicate in many cases damage would be expected. Furthermore, yield loss studies have confirmed significant economic loss on common winter wheat under rain-fed conditions (Elekçioğlu *et al.* 2009), especially under rain-fed post anthesis drought conditions.

There are many possible control options for CCN which have been reviewed in the literature (Nicol and Rivoal 2008) and the most effective is a combination of genetic host resistance with rotation of non-hosts in the cropping sequence. However, in the CAP of Turkey the majority of the wheat production system is a wheat fallow with limited rotation. As a result one of the most useful control measures for this region is the host resistance which will enable the nematode population to be kept below economically damaging thresholds. This approach is seen to be the most cost effective, environmentally friendly and easily accessible. As a result CIMMYT and Turkey in a joint collaborative initiative have established an *in vitro* screening method to screen a range of both winter and spring wheat germplasm from both National and International sources to identify promising levels of resistance.

METHODS

The most effective method to screen for resistance is under controlled greenhouse conditions with mass culture of CCN. Adopting such published methods this work has been successfully initiated in Turkey over the past five years (Nicol *et al.* 2007). As reviewed by Nicol and Rivoal (2008) resistance against the closely related species *H. avenae* has been widely published with six out of the seven published *Cre* resistance genes coming from wild relatives of wheat *Aegilops* genus.

More than 1000 national and international winter and spring wheat lines and cultivars from Turkish national and international (CIMMYT and Turkey-CIMMYT-ICARDA International Winter Wheat Improvement Program) and other international sources were screened in order to determine their resistance against *H. filipjevi* local population TK1 from Haymana. Furthermore, the known published *Cre* genes available in bread wheat background for *H. avenae* resistance were also screened.

In vitro greenhouse screening was used using modifications of existing methods used by other collaborators working on CCN. Screening was conducted under greenhouse conditions at 22-25C with 12 h of light. Seven replicates per line arranged as a randomised complete block design using an optimised screening methodology for CCN. Wheat seeds were germinated and planted in open ended

cylindrical tubes (3 x 13 cm) filled with soil mixture of sand, field soil and organic matter (70:29:1 v/v). One hundred *H. filipjevi* juveniles in 1 ml of water are added after planting and similarly one day later. Nine weeks post inoculation, the plants are harvested and washed. The number of white females are counted per root system and also the number of cysts in the soil used the modified Fenwick can. Two moderately resistant (Sönmez, Silverstar or Katea) and susceptible (Bezostaya 1 and Kutluk) lines were used in each test to compare data from ANOVA.

RESULTS

Repeated screening has identified 27 lines to have resistance as good or better than the known control lines. Of the known published *Cre* genes, *CreR*, *Cre1* and the Milan VPM source - *Cre5* (also possibly *Cre2* and *Cre6* genes) was found to provide some level of moderate resistance, while others tested (*Cre3* and *Cre8*) were found to be ineffective. Further work is necessary to confirm if these published genes, which are effective for the *H. filipjevi* Turkish population, are associated with the same genes or if the resistance is coming from elsewhere. At least two of the identified sources have wild progenitors of wheat (Milan with *Aegilops ventricosa* VPM segment and CROC_1/AE.SQUARROSA(205)//KAUZ/3/LUFER with *Aegilops squarossa* (syn *tauschii*). As a frequency the number of promising durum (tetrapolid) wheat with resistance was much greater than the hexaploid bread wheat (data not presented), suggesting that useful resistance may be associated with the AB genome. Most of the sources presented are in either released cultivars or advanced lines which could easily be used for bread wheat improvement. Several of the sources have also been found to have valuable sources of resistance against root lesion nematode, especially the Iranian landraces (Sheedy and Thompson 2009).

DISCUSSION

Many of the lines identified are in high yielding adapted spring and winter wheats, and several represent Turkish released cultivars, and it is plausible to suggest that these may have been selected under disease pressure. Ongoing work (Sağlam *et al.* 2009), suggests two of the most promising sources of winter bread resistance (Sonmez and Katea-1) have different mechanisms of resistance from each other, and hence may represent new or additional genes. The most promising germplasm which has been identified have been shared with International and National breeding programs in the region including China, India, Iran and Tunisia. Further work is needed to validate this resistance under natural field conditions as well as characterise the sources of resistance.

As Nicol and Rivoal (2008) indicate the effectiveness of designated *Cre* genes depends on both the species of CCN and pathotype. As CCN is a known regional problem in west Asia and north Africa a quarantined laboratory has been established in Turkey at Çukurova University which will enable testing of the most promising resistance material identified in Turkey against *H. filipjevi* to determine if they are effective against populations of CCN from partner countries. Others are welcome to join in this collaboration.

Table 1. Sources of bread and durum wheat resistance to cereal cyst nematode *Heterodera filipjevi* TK1 Haymana isolate from repeated *in vitro* screening under controlled greenhouse conditions in Turkey.

Cross name	*Cre* gene[1]	Accession[2]	Cross number	Source[3]	Type[4]
Spring Bread Wheat					
6R(6D)	*CreR*	30883		A	AL
ID-2150	*Cre2*	20626		S	AL
MILAN	VPM (*Cre5* + ?)	990659	CM75113	CM	RC
SILVERSTAR	*Cre1*	31017		A	RC
AUS GS50AT34/SUNCO//CUNNINGHAM		30799	CMSS99Y05529T-12M-6Y-010M-3SY-0B	CM	AL
AUS GS50AT34/SUNCO//CUNNINGHAM		30798	CMSS99Y05529T	CM	AL
AUS GS50AT34/SUNCO//CUNNINGHAM		30795	CMSS99Y05529T-12M-1Y-010M-2SY-0B	CM	AL
IWA 8604765			PI 628144	I	LR
IWA 8608077			PI 621458	I	LR
SUNCO/FRAME//PASTOR		50785	CMSS99M01589T	CM	AL
Winter Bread Wheat					
CARDINAL/4/KVZ/CUT75/3/YMH//61.1523/DRC		50219	TCI960121	TCI	AL
BEZ//BEZ/TVR/3/KRMN/LOV29/4/KATE/5/MOMT			TE 5446	T	AL
BILINMIYEN96.7		64	F2.96.7	TCI	AL

[1]*Cre* gene is reported against CCN species, refer to Nicol and Rivoal (2008) for further information. [2]TCI – Turkey, CIMMYT (International Maize and Wheat Improvement Centre), ICARDA (International Centre from Research in Dryland Agriculture) accession number for the cross. [3]A, Australia; S, Spain; CM, CIMMYT Mexico; I, Iran; TCI, Turkey CIMMYT ICARDA; T, Turkey. [4]RL, released cultivar; LR, landrace; AL, advanced line.

Table 1. (continued)

Cross name	*Cre* gene[1]	Accession[2]	Cross number	Source[3]	Type[4]
Winter Bread Wheat (continued)					
CROC_1/AE.SQUARROSA(205)//KAUZ/3/LUFER		40377	TCI971290	TCI	AL
F130L1.12/ATTILA		980872	MXTK930038	TCI	AL
LOV41//LI7/LE2062		161	AMJ21159	TCI	AL
TR.DUR/BEZ/3/2*YUBILEINAYA/P49// AKHTYRCHANKA/6/SN64//SKE/2*ANE/3/SX/4/ BEZ/5/JUN/7/BONITO		9059	TCI962283	TCI	AL
MVR27-82//LI7/LE2062		406	AMJ20983	TCI	AL
BAĞCI2002				T	RC
KARAHAN99				T	RC
KATE A-1				T	RC
SONMEZ				T	RC
YAKAR				T	RC
TOSUNBEY				T	RC
Winter Durum Wheat					
C1252				T	RC
KIZILTAN91				T	RC
TARM				T	AL

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IDENTIFICATION AND UTILISATION OF GENES FOR CEREAL CYST NEMATODE RESISTANCE (*HETERODERA AVENAE*) RESISTANCE IN WHEAT: THE AUSTRALIAN EXPERIENCE*

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SUMMARY

The cereal cyst nematode (CCN, *Heterodera avenae*) is an important disease in many wheat-growing regions of the world. For several decades up to and including the 1990s, it caused severe losses in southeastern Australia, where on average an 8% reduction in grain yield has been reported; although yield losses in individual fields can be more than 50%. In the late eighty's the wheat industry in Australia initiated a research program aimed at identifying new sources of CCN resistance, understanding the genetic basis of resistance, and developing efficient means of screening for CCN resistance in wheat breeding programs and carry out targeted breeding to incorporate diverse resistance genes into key cultivars and germplasm. Subsequently, we identified new sources of resistance in *Aegilops tauschii* (genome designation D^tD^t) designated *Cre3*, in *Ae. ventricosa* (D^vN^v) introgression lines designated *Cre6* and potential novel sources of resistance in synthetic hexaploid wheat against the Australian CCN pathotypes (Ha13). Genes encoding nucleotide binding site-leucine rich repeat (NBS-LRR) proteins that cosegregate with the *Cre3* locus cross hybridised to homologues whose restriction fragment length polymorphism patterns were able to distinguish near-isogenic *Cre1* nematode-resistant wheat lines as well as *Cre6 Ae. ventricosa* bread wheat introgression lines. The NBS-LRR gene family derived from the *Cre3* founder locus were used to develop diagnostic markers for diverse CCN resistance genes - *Cre1*, *Cre3, Cre5* and *Cre6* - to facilitate the introgression of CCN resistance from diverse sources into adapted local cultivars. Consequently, marker-assisted selection for CCN resistance is performed routinely in many wheat-breeding programs worldwide notably in

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Australia, CIMMYT and ICARDA. The successful control of the disease for over the past two decades with genetic resistance has resulted in a sharp decline in the incidence of CCN infection in wheat fields. In other regions of the world where CCN remains a major production constraint, identifying new sources of resistance, pyramiding different genes and developing adapted resistant cultivars and replacing the susceptible cultivars is the most sustainable strategy to mitigate potential losses.

INTRODUCTION

Cereal cyst nematode (CCN, *Heterodera avenae*) causes significant economic losses in cereal crops in temperate countries. The effective control of diseases of cereals in particular wheat is critical to maintaining stability in global food supplies. In many cases the availability of genetic resistances are often limited because of the lack of genetic diversity within cultivated species. Resistance is particularly important to farmers in developing countries where food production is less advanced and much of the world's wheat is produced. Resistance to cereal cyst nematode (*H. avenae*) was first reported in barley (Nilsson-Ehle 1920), and four decades later, was also described in bread wheat (Nielsen 1966). The resistance from bread wheat was designated *Cre1*.

DIVERSITY OF RESISTANCE SOURCES

To date, 11 different CCN resistance genes are now catalogued, most of which were incorporated in wheat from wild relatives of wheat (Table 1). Only one of the resistance genes appears not to be pathotypes specific. Pathotypes specificity derives from the gene-for-gene relationship between the host plant resistance gene and corresponding virulence genes in the pathogen. *Cre1* also confers resistance to several European cyst nematode pathotypes. *Cre2* exhibits a high level of resistance to populations of *H. avenae* Ha71 (Spanish), Ha11 (British), and Ha12 and Ha41 (French) but proved ineffective against HgI-HgIII (Swedish) and the Australian Ha13 (Delibes *et al.* 1993, Ogbonnaya *et al.* 2001a). *Cre3* and *Cre6* provide better resistance than *Cre1* against pathotype Ha13 but they are susceptible to the European pathotypes Ha11 and Ha12 (Ogbonnaya *et al.* 2001a). *Cre5* confers partial resistance to French (Ha12 and Ha41) and Australian (Ha13) pathotypes (Rivoal *et al.* 1993, Jahier *et al.* 2001, Ogbonnaya *et al.* 2001a). Wheat cultivars carrying *Cre8*

Table 1. Original source species of designated cereal cyst nematode resistance genes.

Source species	CCN resistance genes	References
Aegilops tauschii	*Cre3*, *Cre4*	Eastwood *et al*. 1991, 1994
Aegilops triuncialis	*Cre7*	Romero *et al*. 1998
Aegilops variabilis	*CreX*, *CreY*	Barloy *et al.* 2007
Aegilops ventricosum	*Cre2*, *Cre5*, *Cre6*	Delibes *et al*. 1993, Jahier *et al*. 1996, Ogbonnaya *et al*. 2001a
Secale cereale	*CreR*	Asiedu *et al*. 1990
Triticum aestivum	*Cre1*, *Cre8*	Slootmaker *et al*. 1974, Williams *et al*. 2003

exhibit partial resistance and tolerance to Ha13; its effect on European pathotypes is unknown. Because of its wider specificity, *Cre1* is the most widely used gene and has been bred into commercial cultivars grown in Australia and Europe. Ogbonnaya and coworkers (2001a) reported that the inhibition of Ha13 nematode reproduction was ranked in the order *Cre6* > *Cre1* > *Cre8* and *Cre5*. They found that *Cre6* was inherited as a single dominant locus.

BREEDING STRATEGIES FOR RESISTANCE TO CEREAL CYST NEMATODE IN WHEAT

Breeding for CCN resistance involves two phases, pre-breeding and incorporation/ transfer of resistance in breeding programs. Pre-breeding often involves the search for and identification of resistance sources, genetic understanding of the nature of resistance and the development of linked molecular markers where possible. This is followed by the incorporation of the resistance into adapted elite cultivars which are then used in breeding programs. The fastest way to improve the resistance of wheat cultivars including elite adapted germplasm is to incorporate diverse sources of resistance into them through limited or repeated backcrossing. The number of backcrosses needed to achieve agronomically similar derivatives with equivalent quality attributes may vary depending upon the donor background (Bariana *et al*. 2007). Non-specific resistance genes, such as *Cre1,* that confer resistance against multiple pathotypes would be the desirable starting point. The pathotypes specific resistance genes are also important and could be deployed by pyramiding diverse sources of resistance genes.

Most Australian breeding programs employ a combination of backcross and topcross methods to combine more than one resistance gene in a single genotype. To transfer two or more effective resistance genes into an adapted cultivar, the better crossing strategy is to cross the resistance sources and then cross the resultant F1 plants with the adapted cultivar. Molecular markers can then be used to select top-cross plants that have desirable agronomic features and carry the targeted resistance genes. Because such plants are expected to be in low frequency, it is desirable to maintain a large family size of about 400, which can be obtained by emasculating and pollinating 20 spikes. Further backcross on selected plants will help restore the characteristics of the recurrent parent (Singh *et al*. 2006). This strategy was adopted to develop wheat germplasm carrying *Cre1*, *Cre3* and *Cre8* genes (Lagudah and Ogbonnaya unpublished data). Ogbonnaya *et al.* (1998) reported the successful marker-assisted pyramiding of CCN resistance genes, *Cre1* and *Cre3,* using marker-assisted selection (MAS). Wheat germplasm lines with the two pyramided genes homozygous for *Cre1* and *Cre3* exhibited a higher level of resistance to the Ha13 pathotype than the two parental single gene lines alone. The level of resistance observed suggested an additive effect of the two genes in the pyramided line.

DEPLOYMENT OF LINKED MOLECULAR MARKERS IN BREEDING FOR CEREAL CYST NEMATODE RESISTANCE

The use of MAS has been advocated as a highly efficient alternative to bioassays because it offers rapid and precise selection of the target gene (Tanksley *et al.* 1989), without the need to wait for phenotypic expression (Peng *et al.* 1999). MAS can

produce reliable results on a single-plant basis and obviates the confounding effects of environment on phenotyping or biological assays.

Previous studies have reported the identification and mapping of CCN resistance genes, *Cre1* in *Triticum aestivum* on 2B (de Majnik *et al*. 2003), *Cre2* in *Ae. ventricosa* on 5Nv) (Delibes *et al*. 1993, Ogbonnaya *et al.* 2001a), *Cre3* from *Ae. tauschii* on 2D, (Eastwood *et al*. 1994, Ogbonnaya *et al*. 2001b), *Cre4* in *Ae. tauschii* (Eastwood *et al*. 1991), *Cre5* from *Ae. ventricosa* on 2A, (Jahier *et al*. 2001), *Cre6* (*Ae. ventricosa* 5Nv) (Jahier *et al.* 1996, Ogbonnaya *et al*. 2001a), *Cre7* in *Aegilops truincialis* (Romero *et al*. 1998) and *Cre8* in *T. aestivum* on 6B (Williams *et al*. 2003). *CreX* and *CreY* identified in *Ae*. *variabilis* accession no. 1 confers resistance against the pathotype Ha12. The effective implementation of molecular markers linked to CCN resistance relies on the diagnostic ability of the marker(s), user-friendly protocols and their utility across diverse genetic backgrounds. Such markers ensure selection of a target gene based on the presence of linked/diagnostic markers. Closely linked DNA markers that have been validated and used in MAS for CCN resistance breeding in Australia are given in Table 2. These widened the range and scope of MAS in breeding for CCN resistance in wheat. In the breeding program of Australian Grain Technologies Pty Ltd, the deployment of markers linked to CCN resistance genes is for population enrichment of complex cross including backcross and/or topcross F1 plants. They are also being

Table 2. Some DNA diagnostic markers linked to cereal cyst nematode resistance genes being deployed by Australian wheat breeding programs.

Gene	Chromosome location	Markers	Reference
Cre1	2BL	M19Cre1F, M19Cre1R	de Majnik *et al*. 2003
Cre3	2DL	Cre3spF, Cre3spR	Ogbonnaya *et al*. 2001b
		gwm301, gwm577	Martin *et al*. 2004
Cre8	6B	stm647, gdm147	Williams *et al*. 2006

used to track the incorporation of CCN resistant genes into adapted elite lines and on early-generation breeding materials to increased the frequency of CCN resistant lines (Martin *et al.* 2004).

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PRELIMINARY INVESTIGATION OF RESISTANCE IN WINTER WHEAT TO *HETERODERA FILIPJEVI* UNDER CONTROLLED CONDITIONS*

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SUMMARY

This study was initiated to explore the process of resistance of four moderately resistant wheat cultivars (Katea, Sönmez, Milan and Silverstar) in comparison with the susceptible check Bezostaya to the Turkish isolate TK1 (Haymana) of cereal cyst nematode *Heterodera filipjevi*. Under replicated controlled conditions, the number of *H. filipjevi* that penetrated and their stage of development were monitored at weeks 2, 3 and 4 post-inoculation. Two and 3 weeks after nematode inoculation, penetration was highest in cv. Bezostaya and nematode development was faster in this cultivar. Nematodes penetrated cvs Katea, Milan and Silverstar but their development was slower when compared with the susceptible cv. Bezostaya.. In this study, the process of resistance in Sönmez was apparently different to that of the other moderately resistant cultivars, in which penetration was highly inhibited. Further investigation of the novelty of resistance mechanism and its genetic control in Sönmez is recommended.

INTRODUCTION

In 2007 world production of wheat were 607 Mt, making it the third most-produced cereal after maize (784 Mt) and rice (651 Mt) (Anon. 2007). Wheat is an important food production crop for Turkey, with annual production of 21 Mt and average yield 2 t/ha (Anon. 2009). As recently reviewed by Nicol and Rivoal (2008), cereal cyst nematodes (CCN) are recognised as biotic constraints of economic importance for

*Sağlam HD, Çobanoğlu S, Wesemael W, Nicol JM, Viaene N, Dababat AA (2009) Preliminary investigation of resistance of winter wheat to *Heterodera filipjevi* under controlled conditions. In 'Cereal cyst nematode: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 172-176. (CIMMYT: Ankara, Turkey)

wheat production in many countries, with the most important species being *Heterodera avenae, H. filipjevi* and *H. latipons*.

The Central Anatolia Plateau (CAP) is considered the main rain-fed cereal production region in Turkey. *H. filipjevi* was recently found to occur in 78% of the soil samples collected on the CAP and yield loss due to damage by *H. filipjevi* reached as much as 47 and 37% in winter wheat cultivars Bezostaya and Gerek, respectively (Elekçioğlu *et al.* 2009).

H. filipjevi is also reported from many other wheat-producing countries including Greece, India, Iran, Norway, Russia, Sweden, Tadzhikistan and USA (Nicol and Rivoal 2008, Smiley *et al*. 2008).

Control of *H. filipjevi* can be achieved through a number of integrated pest management methods as summarise by Nicol and Rivoal (2008). However, the 5 Mha on the CAP of Turkey is predominately a wheat fallow system and the possibility to rotate with non cereal hosts is limited. One of the best methods for control is the use of genetic host resistance which is defined as the reduction in nematode population below economic levels for damage. Resistance is economically, environmentally friendly and very effective when compared to the other control methods. At least nine different resistance genes have been identified in cereals in Australia, France and Spain against the closely related *H. avenae* (Nicol and Rivoal 2008).

Previous screening for resistance has confirmed that two wheat cultivars, Silverstar and Milan, recognised globally for resistance to *H. avenae,* have moderate resistance against *H. filipjevi* TK1 population form Turkey (Nicol *et al.* 2009). Further work screening Turkish national wheat germplasm has identified two Turkish released cultivars, Katea-1 and Sönmez, as providing moderate resistance (Nicol *et al*. 2009).

The objective of this study was to investigate the resistance in four moderately resistant wheat cultivars, Sönmez, Katea-1, Milan and Silverstar, against *H. filipjevi* under controlled conditions, in comparison to the susceptible wheat cultivar Bezostaya.

METHODS

Cultivars examined were the susceptible winter wheat cv. Bezostaya and four moderately resistance (MR) cultivars; two spring wheat cvs Milan and Silverstar and two winter wheat cvs Sönmez and Katea-1. Cysts of a population of *H. filipjevi* (TK1) from a field near Haymana, Turkey (39° 24' 13" N, 32° 37' 14" E) were collected at the end of the season. The cysts were surface sterilised (0.5% NaOCl for 10 min, 3 rinses in sterile water) and used producing inoculum for experiments conducted under control conditions, modified from the methods of Nitao *et al.* (1999).

Seeds were planted into individual tube (17 cm^3) containing a mixture of sterilised sand and river soil (1:1, v/v). One hundred second stage juveniles (J2) of *H. filipjevi* were inoculated into each tube just after germination. Tubes were placed in a glasshouse at 21-22C, 75% of relative humidity, with a supplementary 16 h/day artificial light. To determine nematode penetration, root systems were harvested at

the 2, 3 and 4 weeks after inoculation. The roots were washed and the nematode inside the roots were stained with acid fuchsin as described by Byrd *et al.* (1983). The number of nematodes at each stage of development (J2 to adult) were recorded at each time. Each treatment was replicated 7 times and arranged in a randomised complete block design. Data were analysed using SPSS 17 for Windows and LSD was used for mean comparison. Statistical differences referred to in the text are significant at ($P \leq 0.05$).

RESULTS

Nematode penetration was significantly greater in Bezostaya at week 2 than in other cultivars (Figure 1). However, by week 4 there were no significant differences between cultivars except for the low number in Katea-1 (Figure 1). Among MR cultivars no nematodes were found to penetrate Sönmez until week 4 (Table1). Staining the root system revealed the development of the nematodes over time, which clearly illustrated delayed development in all MR cultivars relative to the susceptible check (Table 1). After 4 weeks, only J3 were found in the MR cultivars except for Katea-1, in which a few J4 were found. Adults were found after only three weeks in Bezostaya (Table 1).

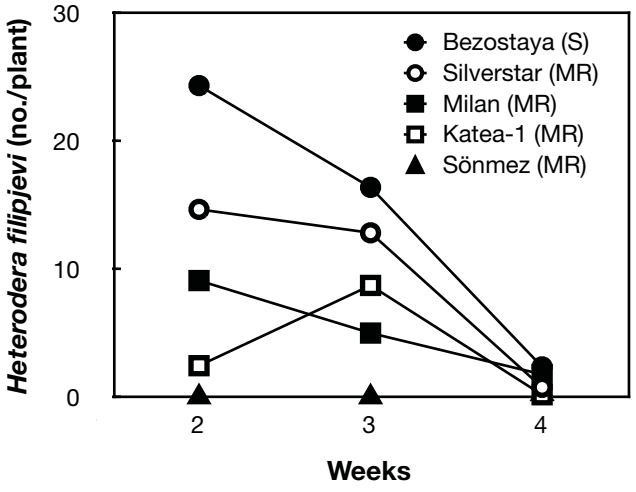

Figure 1. Penetration of *Heterodera filipjevi* in four moderately resistant (MR) and one susceptible (S) wheat cultivars, 2, 3 and 4 weeks after inoculation (n=7).

DISCUSSION

The preliminary results infer a incompatibility reaction in all MR cultivars studied. This reaction is seen by both the lowered initial penetration of juveniles at week 2 and their delayed development over the four weeks compared with susceptible check, cv. Bezostaya. Additionally, Sönmez appears to have different effect on *H. filipjevi* than the other three MR cultivars. In Sönmez juveniles were not found in the roots until week 4. The currently known genetic sources of resistance for *H.*

Table 1. The development stages (J2 to adults) of *Heterodera filipjevi* in susceptible (S) and moderate resistance (MR) wheat cultivars over 4-week time period.

Cultivars	Time (Weeks)		
	2	3	4
Bezostaya (S)	J3-J4	J3 to adult	J4 and adult
Katea-1 (MR)	J2-J3	J3-J4	J3-J4
Milan (MR)	J2-J3	J3	J3
Silverstar (MR)	J2-J3	J2-J3	J3
Sönmez (MR)	none	none	J3

avenae have been reviewed (Nicol and Rivoal 2008, Nicol *et al.* 2009) and both Milan and Silverstar are documented to have *Cre* genes. Further research should confirm if these *Cre* genes are also the key sources of the resistance reaction found with *H. filipjevi*.

Resistance in Sönmez appears to be potentially novel due to its differential reaction relative to the other MR cultivars. This is a commercially released cultivar in Turkey with high yield and adaptability. Further work should be undertaken to confirm these findings under both greenhouse and field conditions. If the resistance is truly novel, then understanding its genetics and mechanism will offer substantial opportunities for additional genetic control through its incorporation in wheat improvement programs.

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USE OF MOLECULAR MARKERS FOR SCREENING WHEAT GERMPLASM FOR CEREAL CYST NEMATODE RESISTANCE GENES (*CRE*) IN SAUDI ARABIA*

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SUMMARY

Cereal cyst nematode (CCN), *Heterodera avenae,* significantly reduces bread wheat (*Triticum aestivum*) yields in the Saudi Arabia. This study aimed to screen wheat lines for resistance genes using specific primers developed for known resistance genes to aid in the development of resistant cultivars of bread wheat. Forty genetically diverse wheat genotypes with and without CCN resistance genes (*Cre*) were sown into a field infested with CCN. Also, microsatellite markers linked to *Cre1, Cre3, Cre5, Cre8, CreX,* and *CreY* genes were used in this study. The results showed that there were significant differences in resistance to CCN among wheat genotypes. Moreover, the results indicated that *Cre3* gene located on chromosome 2DL, provides high levels of resistance to CCN in wheat genotypes using *Xgwm301* marker. Amplification conditions for the *Xgwm301* locus were optimised for marker-assisted selection of *Cre3*-based resistance in Saudi wheat cultivars.

INTRODUCTION

In 1987, the cereal cyst nematode (CCN), *Heterodera avenae,* was reported in wheat in Saudi Arabia (Youssif, 1987). Since then, *Heterodera avenae* has been increasingly detected in Saudi Arabia and is now recognised as a damaging

*Al-Dos AA, Al-Hazmi AS, Dawabah AAM, Abdel-Mawgood AA, Moustafa KA, Al-Rehiayani SM, Al-Otayk S, Motawei MI (2009) Use of molecular marker for screening wheat germplasm for cereal cyst nematode resistance genes (*Cre*) in Saudi Arabia. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 177-182. (CIMMYT: Ankara, Turkey)

pathogen of wheat and barley (Al-Hazmi *et al*. 2001, Al-Rehiayani 2002). CCN is also found in more than 31 countries, spanning the world's wheat growing areas (Eastwood *et al.* 1994). Damage is often more severe in rain-fed cropping areas with soils poor in nutrients. The yield losses in wheat can be as much as from 30 to 70% (Fisher 1982, Eastwood *et al.* 1994). Crop rotation, host resistance (O'Brien and Fisher 1974) and host tolerance (Fisher *et al*. 1981) are the most economically and environmentally sustainable methods of controlling this nematode. CCN also damages barley, oat, rye and related grain and forage crops.

Seven genes for resistance to CCN have been identified in hexaploid wheat and its relatives: *Cre1* in *Triticum aestivum* chromosome 2B (Williams *et al*. 1994), *Cre2* transferred to wheat from *Aegilops ventricosa* (Delibes *et al.* 1993), *Cre3* on chromosome 2D transferred from *Aegilops tauschii* (Eastwood *et al.* 1994), *Cre4* in *A. tauschii* (Eastwood *et al.* 1991), *Cre5* on VPM1 segment of chromosome 2A from *A. ventricosa* (Jahier *et al.* 2001), *Cre6* in *A. ventricosa* 5NV (Ogbonnaya *et al.* 2001), *Cre7* in *Aegilops truincialis* (Romero *et al.* 1998) and *Cre8* on chromosome 6B in *T. aestivum* (Williams *et al.* 2003). CCN resistance genes have also been mapped in rye (6R, Taylor *et al*. 1998) and barley (2H, Kretschmer *et al.* 1997). A linkage disequilibrium study (Paull *et al.* 1998) found an RFLP locus, *Xcdo347*, that was associated with the Festiguay-derived CCN resistance in wheat cvs Molineux, Frame and Barunga. Williams *et al.* (2003) used this RFLP as a starting point to genetically locate, with RFLP markers, the gene *Cre8* which provides CCN resistance (and tolerance) in the cultivar Molineux. *Cre1* confers resistance to several European pathotypes of *H. avenae* as well as the Australian pathotype, albeit with varying degrees of nematode reproduction in different genetic backgrounds. Comparison of *Cre1* with the nematode resistance gene *Cre3*, derived from the diploid D genome progenitor of wheat, *Aegilops tauschii*, shows that both provide resistance to the Australian pathotype, but differ in their specificity to European and Middle Eastern pathotypes (Ogbonnaya *et al.* 2001). *Cre1* and *Cre3* are located on the long arms of chromosomes 2B and 2D, respectively (Eastwood *et al.* 1994, Williams *et al.* 1994). The objective of this study was to screen wheat lines for resistance genes using specific primers developed for known resistance genes to aid in the development of resistant cultivars in bread wheat.

METHODS

Forty genetically diverse wheat genotypes with and without *Cre* genes were used in this study. These included eleven wheat selections from CCN-infested wheat fields (L1-11), eleven breeding lines from CIMMYT (L12-22), two breeding lines from ICARDA (L23-24), nine advanced (F8) lines selected from the wheat breeding program at the Plant Production Department, College of Food and Agricultural Sciences, King Saud University (L25-33), and six CCN-resistant lines from Australian wheat breeding programs (L34-39) and the susceptible, commonly grown local cultivar Yecora Rojo (L40).

Resistance Evaluation

A field experiment was conducted at the Agricultural Research Station, Nadek company, Saudi Arabia, during 2008/09 in fields infested and uninfested with CCN. Forty wheat lines and cultivars were sown on 24 December 2008, with a seeding rate of 140 kg/ha. The plot size was 3 x 0.8 m^2 with row to row spacing of 20 cm.

The recommended fertiliser for wheat in Al-Qassim region, Saudi Arabia of NPK 200, 200 and 100 kg/ha, respectively, was applied. A randomised complete block design with three replicates was used. One month before harvest, plant samples were carefully collected by selecting an area of 0.8 m^2 within each plot. The plants selected were carefully removed, keeping intact as much of the root systems as possible. Soil around the roots was gently washed away with a stream of water by over a 250 µm sieve. The washed root systems were then examined for the presence of immature white females. The number of white females/plant was determined. Plants having more than 3 females/plant were designated as susceptible (Mathur *et al.* 1974, Ireholm 1994), while those having up to 3 females/plant were designated as "unknown" until they would be re-evaluated again under controlled conditions.

DNA Extraction

Frozen young leaves (500 mg) were ground to a powder in a mortar with liquid nitrogen. The powder was poured into tubes containing 9 ml of warm (65C) CTAB extraction buffer (Sagahi-Maroof *et al.* 1984). The tubes were incubated at 65C for 60-90 min. Then 4.5 ml chloroform/octanol (24:1) was added and tubes were rocked to mix for 10 min, and centrifuged for 10 min at 3200 rpm. The supernatants were pipetted off into new tubes and 6 ml isopropanol was added. After 60 min, the tubes were centrifuged for 10 min and the pellets obtained were put in sterile Eppendorf tubes, containing 400 µl of TE buffer of a pH 8.0 (10 mM Tris-HCl, pH 8.0 and 1.0 mM EDTA, pH 8.0). The DNA extracted and stored at -20C until used.

***Cre* Gene Amplification**

Segments of the *Cre* genes were amplified with a range of primers, these and the PCR cycling used are detailed in Table 1. PCR amplification was performed using a thermal cycler (Thermolyne Amplitron). Amplification was performed in 25 µL reaction volume, containing 1x Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM $MgCl_2$) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 25 pmol primer and 50 ng of total genomic DNA. The PCR products were separated by electrophoresis in 1.5% agarose using TBE buffer and detected by ethidium bromide staining.

RESULTS AND DISCUSSION

Resistance

All the tested genotypes (Figure 1) were found to be susceptible (females/plant > 3) to *H. avenae* (Hail population), except seven advanced lines (L27, L28, L28, L29, L30, L31, L32 and L33) from the wheat breeding program at the Plant Production Department, College of Food and Agriculture Sciences, King Saud University, and three genotypes (L7, L9 and L11) from CCN-infested fields. It could be noted that wheat genotype L11 was the most resistance genotype with no CCN females found (Figure 1). The local cv. Yecora Rojo had 23 females/plant. Previous reports of *H. avenae* reproduction in wheat worldwide are numerous (Meagher 1977, Al-Hazmi *et al.* 1994, Dhawan 1988, Holdeman and Watson 1977, Ibrahim 1989). In Saudi Arabia, Al-Hazmi *et al.* (1994) also reported the susceptibility of eight barley and wheat cultivars, including cv. Yecora Rojo. The ability of the local CCN infect and reproduce on all the tested wheat cultivars and lines herein, especially cv. Yecora

Table 1. Primer (F, forward and R, reverse) names, sequences and chromosomes (Chr.), and PCR cycling conditions for the amplification of specific *Cre* genes.

Gene	Primers	Primer sequence (5′-3′)	Chr.	PCR cycling
Cre1	*G035*	F:TGCAGCCTAGCCTTCTCCCCA R:GTAGCTTCATATATATGGAAC	2B,2BL	1×95C 30 s, 38×(94C 30 s; 50C 30 s; 72C 90 s), 1×72C 10 min 1×10C hold
	Crecon	F:ATCTGATCAACTTGCGGCAT R:ACTCTGACTCCGATTCCAAG		
Cre3	*Xgwm301-2D*	F:GAGGAGTAAGACACATGCCC R:GTGGCTGGAGATTCAGGTTC	2DL	1×95C 30 s, 38×(94C 30 s; 58C 30 s; 72C 90 s). 1×72C 10 min 1×10C hold
Cre5	*Xgwm140*	F:ATGGAGATATTTGGCCTACAAC R:CTTGACTTCAAGGCGTGACA	2AS	1×95C 30 s, 38×(94C 30 s; 55C 30 s; 72C 90 s), 1×72C 10 min, 1×10C hold
Cre8	*Xgwm147-6B*	F:CAAACAAGGTGGGTTCACTG R:TTTTTGAGTTCAACGGAGAC	6BL	1×95C 30 s, 38×(94C 30 s; 50C 30 s; 72C 90 s), 1×72C 10 min 1×10C hold
CreX	*Xgwm636-2A*	F:CGGTAGTTTTTAGCAAAGAG R:CCTTACAGTTCTTGGCAGAA	2AS	1×95C 30 s, 38×(94C 30 s; 50C 30 s; 72C 90 s), 1×72C 10 min 1×10C hold
CreY	OPY16	GGGCCAATGT	3BL	1×95C 3 min, 45×(94C 60 s; 36C 60 s; 72C 90 s), 1×72C 10 min 1×10C hold

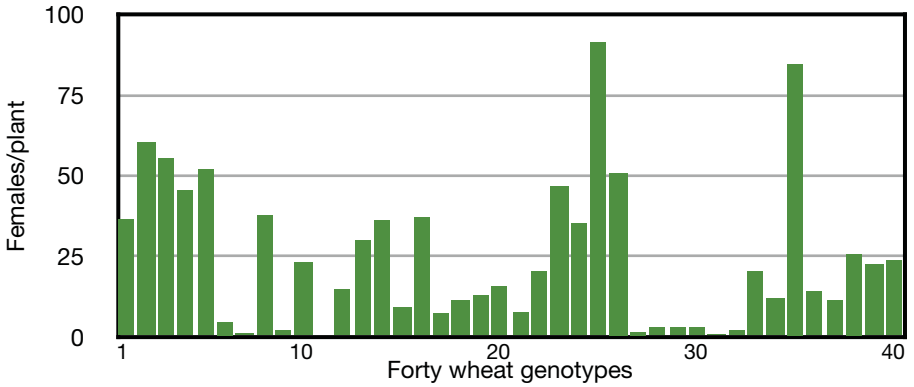

Figure 1. Mean white females of *Heterodera avenae*/plant for 40 wheat genotypes.

Rojo, which is the principal cultivar grown in the Saudi wheat fields (90%), make the search for resistant or tolerant wheat cultivars is of great necessity.

Cre genes

Microsatellite markers linked to *Cre1, Cre3, Cre5, Cre8, CreX,* and *CreY* genes were used in this study. The results showed that *Cre3* gene located on chromosome 2DL, provides high levels of resistance to CCN in wheat genotypes using *Xgwm301* marker (Figure 2). We detected significant association between CCN resistance with *Xgwm301* marker on chromosomes 2DL which explained 27% of the phenotypic variation. Therefore, amplification conditions for the *Xgwm301* locus were optimized for marker-assisted selection to identify *Cre3* based CCN-resistant in Saudi wheat cultivars (Martin *et al.* 2004). Previous studies have identified and mapped CCN resistance genes *Cre1* to *Cre8,* as detailed in the Introduction. Ogbonnaya *et al*. (2001) using the gene-based STS marker, Cre3sp on chromosome 2DL, showed the most significant association, explaining 23% of the phenotypic variation in CCN resistance.

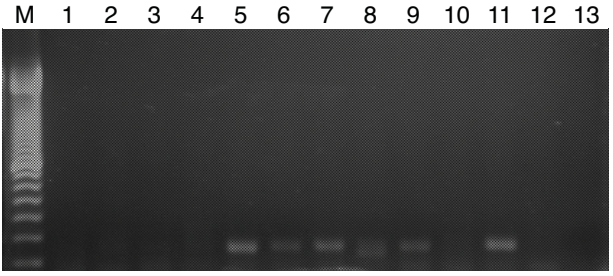

Figure 2. Polymorphism revealed using SSR primers (Xgwm301) to amplify genomic DNA purified from the tested wheat cultivars and lines (L1-13). M lane is 1 Kbp ladder DNA marker.

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EPIDEMIOLOGICAL STUDIES ON THE MEDITERRANEAN CEREAL CYST NEMATODE, *HETERODERA LATIPONS*, ATTACKING BARLEY IN JORDAN*

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SUMMARY

The influence of the root leachate, temperature, soil moisture, soil type and disturbance on Jordanian populations of the Mediterranean cereal cyst nematode, *Heterodera latipons,* was investigated. Results showed that exudates of the host was not essential for egg hatching. The temperature and moisture effects varied with the nematode activity (hatching, penetration, development and reproduction). Hatching occurred in tap water at 10-25C with an optimum temperature at 10C. At 10, 15 and 20C, the second stage juveniles penetrated the barley roots, while the maximum penetration occurred after two weeks at 15C. However at 25C, the nematode failed to penetrate. Optimal soil moisture and temperature for penetration was found to be 20% and 15C, respectively. Nematode development within barley roots was fastest at 20C over a range of moisture from 10-20%. However, 15% soil moisture at 15C was optimal for both development and reproduction. At 10, 15 and 20C it took 85, 49 and 42 d from the penetration to the appearance of young females, respectively. Overall reproduction of the nematode increased within increasing sand content of the soil. Soil disturbance resulted in increased number of cysts produced in barley roots.

INTRODUCTION

In Jordan, barley production is insufficient the country's needs and hundreds of tons are imported annually. Increasing barley production could increase animal production as it is usually used as animal feed. However, one of the obstacles to barley productivity in Jordon is damage caused by plant parasitic nematodes. The

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cereal cyst nematodes, *Heterodera avenae* and *H. latipons* (Mediterranean cyst nematode) are the most common species of cyst nematodes reducing production of barley (Meagher 1977, Philis 1997, Sikora 1988). In Jordan, *H. latipons* was found in different barley and wheat fields (Abed *et al.* 2004, Yousef and Jacob 1994). The severity varied from moderate to high. An early study reported that nematode populations and the host effects disease severity (Abed *et al.* 2004). In this investigation we aimed to study the effect of root exudates, temperature, soil moisture, soil type and disturbance on *H. latipons* from Jordan

METHODS

Effect of Soil Temperature and Root Exudates on Hatch

Ten pots filled with 500 g soil were each planted with four seeds of barley cv. Rum. Collections of leachates were made two weeks after shoot emergence (Williams and Beane 1979). The pots were not watered during the 24 h before collection of leachate. A total of 100 ml distilled water was poured into each pot. After 1 h, an additional 300 ml was added and leachate was collected in beakers.

Cysts were incubated in barley leachate or the tap water at 10, 15, 20 and 25C. Treatments were replicated three times and arranged in a completely randomised design. Emerged juveniles were counted and removed at 48-h intervals. After 4 weeks cysts were crushed individually and total J2 and eggs were counted and percentage of hatching estimated. ANOVA was performed and means were separated using LSD (Cochran and Cox 1957).

Effect of Soil Temperature on Penetration and Development

Two seeds of barley cv. Rum were sown into 20-ml plastic vials containing sterilised sandy soil. Eighty vials were prepared and were kept for one week at 25C for seed germination. After germination, seedlings were inoculated with 50 J2/vial. Immediately, after inoculation, every 20 vials were transferred to the corresponding temperature (10, 15, 20 or 25C) chamber with 12-h photoperiod. Two vials from each treatment were harvested weekly at each temperature level, and roots were then washed in water and stained with acid fuchsin. The stained roots were pressed between two glass Petri dishes and the juveniles and their respective stages in the roots were identified and counted. Per cent penetration was calculated from the first and second week samples. The time required for each developmental stage was recorded at each temperature to determine the length of the life cycle.

Effect of Soil Type on Reproduction

Dry, uninfested clay loam soil from Ar-Ramtha was fractionated and used to reconstruct different soil types. Four soil types were constructed were: sand (100, 0 and 0% of sand, silt and clay), sandy loam (75, 15, and 10%), loam (50, 29 and 21%), clay loam (26, 43 and 31%). A total of 500 g of each soil type were filled in 10-cm pots and thoroughly soaked for 2 d. Five seeds of barley cv. Rum were sown in each pot and incubated at 20C for one week till germination. A total of 500 J2 and eggs were added into each pot. The pots were then planted with barley cv. Rum seeds in a constant temperature chamber at 15C with 12-h photoperiod. The experiment was arranged in a completely randomised design with five replications.

Ten weeks after sowing, the number of cysts and average eggs per cyst were determined for each soil type. Data were analysed as described above.

Effect of Soil Disturbance on Reproduction

Undisturbed soil cores were collected from a field previously planted with barley in the Ar-Ramtha area. Cores were randomly assigned to two treatments, disturbed and undisturbed. The initial population density was determined (Goodey 1963). Disturbance was achieved by pressing the cores through steel mesh. Five seeds of barley cv. Rum were sown into each pot. Treatments were replicated four times and arranged in a completely randomised design. Pots were placed for ten weeks in a temperature controlled growth chamber at 15C with 12 h photoperiod. At harvest, numbers of cysts and average eggs per cyst were determined. Plant height, root and shoot weighs (fresh and dry) were recorded. Data were analysed as described above.

RESULTS

Effect of Temperature and Root Leachates on Hatch and Emergence

Root leachate treatment made no significant difference to hatching. Emergence of juveniles from cysts incubated for 1 month at 10C in water was the highest (33%) followed by those incubated at 15C, 20C and 25C, respectively (Table 1).

Table 1. Effect of temperature and root exudates on cumulative hatching and emergence of juveniles from cysts of *Heterodera latipons.*

Treatments	Hatching (%)
10C + water	33.0 a
10C + root leachate	27.7 ab
15C +water	22.0 bc
15C + root leachate	18.3 cd
20C + water	20.3 bcd
20C + root leachate	18.0 cd
25C + water	13.7 cd
25C + root leachate	12.0 d
LSD, P=0.05	9.09

Values are mean of three replicates. Mean in a column followed by the same letter are not different (P<0.05) according to Fisher's protected LSD.

Effect of Soil Temperature on Penetration and Development

The J2 of *H. latipons* penetrated barley roots at 10, 15 and 20C but not at 25C. Nematode penetration was greatest at 20C after 1 week with 17% penetration. While after 2 weeks, the highest significant penetration of 35% occurred at 15C (Table 2).

The development for all stages was faster at 20C than at 10 or 15C (Table 3). It took 85, 49 and 42 d from J2 penetration to appearance of females at 10, 15 and 20C. At 25C the J2 failed to penetrate the root (Table 3).

Effect of Soil Type on Reproduction

The total number of cysts formed was higher in 100 and 75% sand than with 25% sand. No significant differences were observed in number of cysts formed at 100, 75

Table 2. Penetration of *Heterodera latipons* into roots of barley cv. Rum at 7 and 14 days after inoculation at four constant temperatures (n=4).

Temperature (C)	Penetration (%)	
	7 days	14 days
10	5	11
15	11	35
20	17	24
25	0	0

Per cent penetration = total number of juveniles in roots/number of juveniles added*100.

Table 3. Cumulative days required to complete various developmental stages of *Heterodera latipons* on barley cv. Rum at different temperatures.

Developmental stage	Cumulative days required		
	10C	15C	20C
J3	20	14	10
J4 male	35	27	20
J4 female	42	30	24
Male	65	37	30
Female	72	42	30
Swollen white females	85	49	42

and 50% sand. Furthermore, there were significant differences in number of eggs produced per cyst between 100 and 25% sand (Table 4).

Effect of Soil Disturbance on Reproduction

Average eggs per cyst and cysts produced in barley roots in disturbed soil cores were significantly higher than those in undisturbed cores (Table 4). Results also indicated that plant height, and shoot and root dry weights were significantly lower in disturbed than undisturbed soil cores. However, fresh weights of both roots and shoots were not effected (Table 5).

DISCUSSION

There were no significant differences in percentage of hatching of *H. latipons* J2 between root leachate and tap water at the four testing temperatures. Philis (1999) had previously suggested that there might be no requirement for root exudates for hatching of *H. latipons* since many juveniles were found in the soil 1 week before plant emergence.

The emergence of the J2 *H. latipons* from cysts incubated at 10, 15, 20, and 25C, with an optimum emergence at 10C were similar to those of the closely related species *H. avenae* (Banyer and Fisher 1971, Fushtey and Johnson 1966, Zancada and Sanchez 1988, Farivar-Mehin and Shakeri 1994).

Table 4. Effects of soil type on cyst and eggs numbers produced by *Heterodera latipons* in barley cv. Rum.

Soil type	Cysts	Eggs/cyst	Rf[1]
Sand (100% sand)	62.3 a	143.8 a	17.8
Sandy loam (75% sand)	55.0 a	129.3 ab	14.2
Loam (50% sand)	30.0 ab	126.0 ab	7.6
Clay loam (25% sand)	17.5 b	122.0 b	4.3
LSD, P=0.05	33.4	23.0	-

Rf, reproduction factor = final/initial population; Means (n = 4) in columns followed by the same letter are not different (P<0.05) according to Fisher's protected LSD.

Table 5. Effect of soil disturbance on reproduction of *Heterodera latipons* in barley in soil cores collected from no-till barley field plots at Ar-Ramtha.

Parameters	Disturbed	Undisturbed	CV	P value
Cyst/pot	2813	176	3.3	0.003
Egg/cyst (Pf)	180	127	11.9	0.03
Fresh shoot (g)	12.68	20.23	32.6	0.14
Dry shoot (g)	3.62	6.52	19.6	0.03
Fresh root (g)	2.1	2.9	16.6	0.08
Dry root (g)	0.63	1.09	13.7	0.01
Plant height (cm)	41.5	52.2	9.0	0.03

CV, coefficient of variation; *Pf,* eggs+J2/pot (1500 g soil) at the end of the experiment; n = 4.

The penetration of J2 of *H. latipons* occurred at 10, 15 and 20C, but not at 25C which agreed with the findings of Mor *et al*. (1992). In their work, the J2 of *H. avenae* and *H. latipons* penetrated barley and wheat roots at 6, 12 and 18C, but failed to penetrate at 24C. In contrast, the penetration of J2 of *H. cajani* in pigeon pea roots was greatest at 25C, with no penetration at 10C (Singh and Sharma 1994).

The time required for the *H. latipons* collected from Ar-Ramtha to complete its life cycle was 85, 49 and 42 d at 10, 15 and 20C respectively. Mor *et al*. (1992) mentioned that *H. avenae* and *H. latipons* needed 4 months at 6C from J2 penetration to the appearance of females and 80 d at 12C, while at 18C it took only 40 d. Philis (1999) reported that under field conditions in Cyprus, the white females of *H. latipons* were observed 68 d from sowing.

The number of cysts produced on barley cv. Rum increased with increasing sand content. Meagher (1977) reported that there was a consistent association of increased disease severity caused *by H. avenae* with lighter soil types.

Results showed that more cysts formed in plants grown in disturbed than in undisturbed soil cores. These findings were in agreement with those of Young (1987). This increase of cysts in disturbed soil cores might be due to the increased J2 movement and penetration, or increased oxygen supply to the roots ensuring more females developed and survived to maturity.

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CYST NEMATODE GENOMICS AND FUNCTIONAL ANALYSIS OF *GLOBODERA PALLIDA* EFFECTORS*

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SUMMARY

The *Globodera pallida* genome sequencing project will allow identification of the full complement of genes which will underpin future studies on this nematode. Functional studies on *G. pallida* effectors, which may induce the formation of the feeding site or suppress host defence responses, are a particular focus for our group. A large gene family of effectors has been identified that may suppress host defences. Some of these proteins are targeted to the plant nucleus. New sequencing technology means that sequencing of other cyst nematode genomes is a realistic goal providing opportunities for comparative genomics studies.

INTRODUCTION

The genome sequencing project for the potato cyst nematode *Globodera pallida* is now well underway. This project is a collaborative venture between Leeds University, SCRI, Rothamsted Research and the Wellcome Trust Sanger Institute. The project, which started in 2008, is scheduled to be finished by 2011. Details of the progress on the *G. pallida* sequencing project can be found at http://www.sanger.ac.uk/sequencing/Globodera/pallida/.

The *G. pallida* genome sequencing project is being greatly facilitated by the availability of new sequencing technology, including 454FLX and Solexa Illumina. A combination of genomic DNA and transcriptome sequencing is being used to generate the sequence and to identify transcribed regions of the genome. These techniques will allow the full complement of *G. pallida* genes to be identified. This information will underpin future studies on this nematode.

The *G. pallida* genome sequence will be completed at an opportune moment as two root knot nematode genomes have recently been published (Abad *et al*. 2008,

*Jones JT Chapman S Cock PJ Blok V (2009) Cyst nematode genomics and functional analysis of *Globodera pallida* effectors. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 189-190. (CIMMYT: Ankara, Turkey)

Opperman *et al*. 2008). The availability of these sequences, and those of other nematodes, will aid gene finding and will allow comparisons of the genomes of cyst and root knot nematodes.

FUNCTIONAL ANALYSIS OF NEMATODE EFFECTORS

The focus of the SCRI group is on functional characterisation of the secreted proteins – effectors – that mediate interactions with the host. Cyst nematodes are biotrophic pathogens and therefore need to suppress host defences in order to complete their life cycles. Effector proteins are likely to be responsible for this suppression of host defences as well as inducing the production of the feeding site. The availability of the full gene complement for *G. pallida* means that the focus is currently on development of tools for functional analysis of effectors. We have developed a high-throughput system for analysing subcellular localisation of nematode proteins in plants and have used it to examine localisation of various members of a large gene family of effectors. In addition, we have developed tools for identifying nematode effectors that suppress basal defences or the hypersensitive response in plants. Cell biology tools have also been developed that will allow a high throughput screen of *G. pallida* effectors for those that induce changes in plant physiology associated with the earliest phases of feeding site induction.

FUTURE PROSPECTS

New sequencing technology means that sequencing of other cyst nematode genomes is a realistic goal. Costs of sequencing have declined dramatically and will continue to fall in the foreseeable future. A strong argument can therefore be made for including a cereal cyst nematode genome sequence at the core of any future collaborative effort on this nematode, as long as sufficient bioinformatics support can be secured for the project. A genome project will undoubtedly provide scientific advances but also offers the opportunity for the development of new control strategies. Additional cyst nematode sequences will also provide opportunities for comparative genomics studies. Such analysis may shed light on to how parasitism and host specificity has evolved in the Nematoda.

ACKNOWLEDGMENTS

The *G. pallida* genome sequencing project is a collaboration between Leeds University, SCRI, Rothamsted Research and the Wellcome Trust Sanger Institute. Principal contacts at each site are: Dr P. Urwin (Project PI), Leeds University; Prof J Jones and Dr V. Blok, SCRI; Prof. B. Kerry, Rothamsted Research, and; Dr M. Berriman, Wellcome Trust Sanger Institute.

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TOWARDS DEVELOPING TRANSGENIC RESISTANCE TO NEMATODES IN WHEAT*

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SUMMARY

Research is in progress to use biotechnological tools to aid development of new forms of diagnostics and resistance to nematodes in wheat. For diagnostics, protein profiling by MALDI-TOF mass spectroscopy has been used to identify a range of nematode species, including seed gall, root-lesion and stem nematodes. Nematode species, and in some cases, races, can be identified and differentiated on the basis of characteristic protein biomarkers. A reproducible assay system for compounds that affect root-lesion nematode replication, based on carrot mini-discs, has been developed. Genomic studies have also been initiated to generate cDNA libraries and genomic sequences of root lesion and cereal cyst-nematodes. For new forms of genetic resistance to nematode pests of cereals, molecular approaches are in progress to identify new gene targets, generation of potential resistance-conferring gene constructs and their introduction into wheat by particle bombardment followed by regeneration and challenge to identify nematode resistant wheat plants.

INTRODUCTION

Western Australia (WA) is the largest producer of wheat in Australia, with about 95% of the total crop exported. The cereal cyst nematode (CCN), *Heterodera avenae*, and root lesion nematodes (RLN), *Pratylenchus* spp., together are responsible for annual estimated losses of up to 15% of the wheat crop in WA. To address this issue, researchers in plant nematology within the Plant Biotechnology

*Jones MGK, Rana J, Fosu-Nyarko J, Zhang JJ, Perera M, Chamberlain D (2009) Towards developing transgenic resistance to nematodes in wheat. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 191-194. (CIMMYT: Ankara, Turkey)

Research Group at Murdoch University, together with NemGenix Pty Ltd (a company located at Murdoch University), are undertaking a number of studies in nematode diagnostics, nematode genomics and genetic resistance to nematodes.

METHODS

The methods used to prepare nematodes for protein profiling by MALDI-TOF mass spectroscopy are provided in Perera *et al*. (2005, 2009).

Mini carrot disc assays: in a laminar flow bench, clean carrot storage roots, stored at 4C, were surfaced sterilised by immersion in 70% ethanol for 5 min followed by 1% v/v commercial bleach (12.5% NaOCl) for 20 min, then washed 5 times in sterile distilled water. Using a sterile 25 mm diameter cork borer, 10 mm long discs were prepared and plated in culture dishes with half strength MS/B5 medium solidified with 0.7% agar. The mini-discs were pre-cultured for 2 weeks to ensure sterility and to start callusing, then inoculated with 50 sterile RLN in the dark at 25C. Nematodes were extracted from the discs by maceration and filtration at weekly intervals and counted. The approach to develop new resistance genes was essentially as described by Yadav *et al.* (2006).

Production of transgenic wheat plants was carried out essentially as described by Weeks *et al.* (1993), using a helium inflow particle gun. Complementary DNA (cDNA) libraries of CCN and RLN were made by standard techniques (Sambrook *et al.* 2002).

RESULTS

Protein profiles can be generated rapidly both from extracted and intact nematodes using MALDI-TOF mass spectrometry. Typical protein profiles of *Anguina tritici*, *Anguina funesta* and *Meloidogyne javanica* are provided in Figure 1. Five protein peaks of m/z 3328_0.1%, 3968_0.1%, 5163_0.1%, 8175_0.1%, and 8242_0.1% were consistently found for *A. tritici*, and peaks of m/z 4488_0.1% and 6289_0.1% for *A. funesta*. For comparison, for *M. javanica*, characteristic peaks of m/z13 498_0.1%, 15 731_0.1%, 19 176_0.1%, 20 116_0.1%, and 21 232_0.1% were obtained.

Mini-carrot Disc Assays for RLN Replication

The carrot mini-disc assay system for quantifying effects of test compounds and treatments on RLN is shown in Figure 2a, and a typical growth curve derived from the system is shown in Figure 2b.

Production of Transgenic Wheat

Following particle bombardment with different synthetic gene constructs, a series of transgenic wheat plants have been regenerated. The system is shown in Figure 3.

Genomics Studies on CCN and RLN

Work is in progress to generate cDNA libraries and genomics sequences from CCN and RLN.

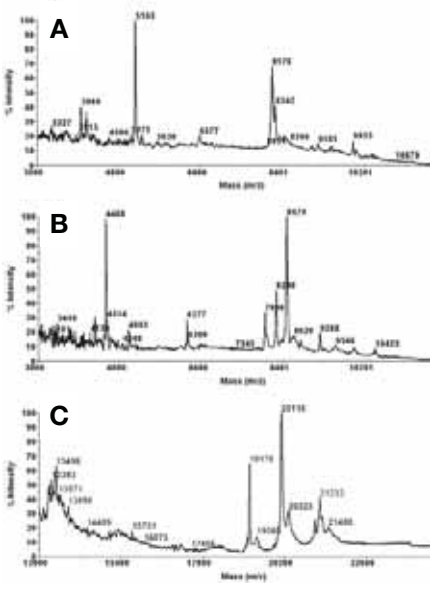

Figure 1. Diagnostic protein profiles of plant parasitic nematodes A, *Anguina tritici*; B, *Anguina funesta* and C, *Meloidogyne javanica*, generated on Voyager DE PRO mass spectrometer in linear mode (Perera *et al*. 2005).

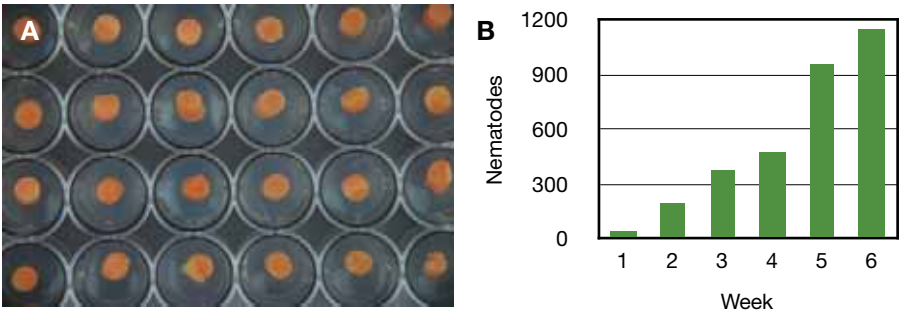

Figure 2. Carrot mini-disc system (A) and the time course of replication of an inoculum of 50 *Pratylenchus thornei* from wheat (B).

DISCUSSION

We have developed new techniques to identify plant parasitic nematodes using MALDI-TOF mass spectrometry and an assay system to quantify effects of treatments on RLN in culture. We are now focusing on a transgenic approach to develop nematode resistance in wheat. Part of that involves the generation of cDNA libraries and genomic sequences of target nematodes, and this work is in progress. Another part of this work involves establishing a pipeline to generate transgenic wheat plants and this aspect has been achieved.

Transgenic crops are now deployed on about 130 Mha worldwide, although currently restricted to four major crops (soybean, maize, cotton and canola). It can be predicted that there will be rapid deployment of transgenic rice in China and following this, the deployment of transgenic wheat can be expected. To anticipate this event we are developing a system to generate wheat plants resistant to major nematode pests.

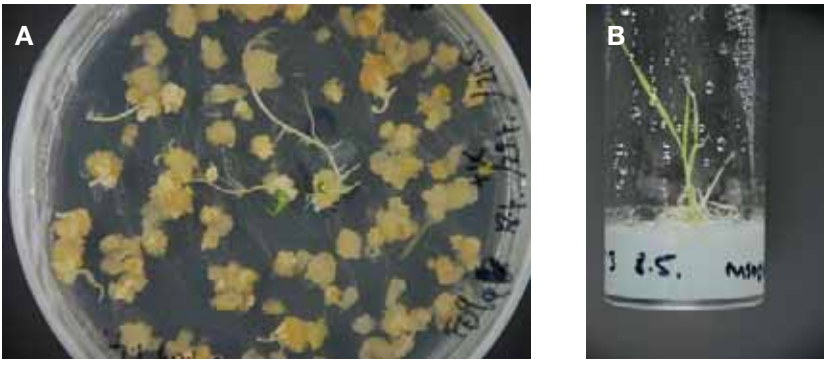

Figure 3. Bombarded wheat cultures selected for regenerating shoots (A) and a transgenic wheat plant (B).

ACKNOWLEDGMENTS

We thank the Australian Research Council for financial support for projects on nematode diagnostics, Murdoch University Institute for Crop and Plant Sciences for small grant support, and Vivien Vanstone (Department of Agriculture and Food WA) for supplying authenticated species of RLN and other nematodes.

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CHROMOSOMAL AND CYTOLOGICAL LOCALISATION OF THREE PEROXIDASES INDUCED BY CEREAL CYST NEMATODE IN WHEAT*

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SUMMARY

In a previous work the characterisation and the alignment of twenty peroxidase genes together with phylogenetic clustering with peroxidases from other plant species showed that these enzymes fall into seven different groups (designated *TaPrx108* to *TaPrx114*). These represent peroxidases secreted to the apoplast by a putative N-terminal peptide signal. Three groups of peroxidase genes (*TaPrx111*, *TaPrx112* and *TaPrx113*) were found to be greatly induced by the cereal cyst nematode *Heterodera avenae* in the H-93-8 resistant wheat line. *In situ* hybridisation analyses of genes belonging to these groups revealed a strong signal of these peroxidases in cells close to the vascular cylinder and parenchyma vascular cells when challenged by nematodes. In particular, parenchyma cells, where the nematode starts its feeding, were hyper-reactive to some probes belonging to *TaPrx112* and *TaPrx113* groups. These findings suggest that wheat apoplastic peroxidases may have a role in the defence response. *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* peroxidases were characterised and their sequences were used to design specific pair primers from each group. Chromosomal analyses by PCR marker mapping and using Sears's aneuploid lines of wheat cv. Chinese Spring indicated that these peroxidases are located on the short arm of the chromosome 2B and that they may be linked each other.

*Simonetti E, Veronico P, Melillo MT, Bleve-Zacheo T, Delibes A, Fè Andrés M, López-Braña I (2009) Chromosomal and cytological localisation of three peroxidases induced by cereal cyst nematode in wheat. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 195-199. (CIMMYT: Ankara, Turkey)

INTRODUCTION

Plant peroxidases (EC 1.11.1.7), often designated as Class III peroxidases (Welinder 1992), are heme containing proteins widely distributed in the plant kingdom. They are members of a large multigenic family, with 138 members in rice (Passardi *et al.* 2004) and 73 members in *Arabidopsis* (Tognolli *et al.* 2002). In contrast to several other gene families, peroxidase genes are twice as numerous in rice as in *Arabidopsis*. This large number of genes resulted from various duplication events by different mechanisms (Zhang 2003); unequal crossing-over, various transposition events, duplication of large chromosome segments or polyploidisation. This is reflected by the distribution of the peroxidase loci, in clusters of closely homogeneous genes, among the 12 rice chromosomes (Passardi *et al.* 2004). Peroxidase genes arranged in tandem have also been found in many other plant species such as horseradish (Fujiyama *et al.* 1988), wheat (Båga *et al.* 1995) and tomato (Roberts and Kolattukudy 1989).

Peroxidase isozymes in hexaploid wheat have been extensively studied and several homologous sets of loci controlling peroxidases have been identified (Bosch *et al*. 1987, Liu *et al*. 1990). Recently, twenty wheat peroxidase genes were shown to fall into seven groups (*TaPrx108-Taprx114*) (Simonetti *et al*. 2009). The purpose of the present investigation was to determine the chromosomal location of *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* peroxidase genes, belonging to *TaPrx111*, *TaPrx112* and *TaPrx113* groups, found to be greatly influenced by nematode attack. In addition, their relationship, if any, with previously described loci was also established.

METHODS

In situ hybridisation experiments were carried out on four-day *H. avenae* infected and uninfected root cross sections from H-93-8 wheat line as described by Jackson (1991) with some modifications as reported by Simonetti *et al.* (2009). The specific cDNA fragments spanning 3' UTRs of genes *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* were obtained by RT-PCR and cloned into pGEM-T Easy vector (Promega). Sense and antisense digoxygenin labelled riboprobes were produced by *in vitro* transcription of linearised plasmid DNA using T7 and SP6 RNA polymerase, respectively, according to manufacturer's instructions.

Total DNA, from leaves of two-week old nulli-tetrasomic and ditelosomic series of *Triticum aestivum* cv. Chinese Spring (Sears 1954, 1966), was isolated using the method described by Taylor and Powell (1982). Primers for the polymerase chain reaction (PCR) were designed to selectively amplify the different peroxidase genes (Table 1).

Table 1. Primer pairs employed for chromosomal location of peroxidase genes in aneuploid line of wheat cv. Chinese Spring.

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
TaPrx111-A	GGATCTACGAGAAATATGCCG	GAATTCGTTACACATGTGGACAG
TaPrx112-D	AGCTGTGTCCTATCTAACAAGCT	CCACCAAGAAATTAAGTACGG
TaPrx113-F	AAGAAGTGCAGGTAGCTAACCA	CATACGTATAGTGTTCAGCATTCAG

RESULTS

Localisation of *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* Transcripts after Nematode Infection

In situ hybridisation on serial sections of uninfected and 4 day-nematode infected wheat roots was performed to analyse the localisation of *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* transcripts and to evaluate their response to *H. avenae* infection. In resistant infected roots each probe gave positive results, by means of brown staining of cells injured by nematode migration. The most reactive sites resulted to be the cortical cells penetrated by the nematode and endodermal and pericycle cells injured by nematode feeding action. In particular a faint signal, only restricted to cells broken by the juvenile entrance, and suggesting a wounding response, was observed in sections hybridised with the probe corresponding to *TaPrx111-A* gene (Figure 1a). In contrast, the probe corresponding to *TaPrx112-D* gene gave heavy staining (appearing as amorphous stained aggregates) of cortical, endodermal and vascular parenchyma cells directly injured by the nematode (Figure 1b). *TaPrx113-F* transcript was found to be located in all cortical cells challenged by nematode penetration (Figure 1c). Sections of uninfected roots did not show any signal whatever probe was used (Figure 1d).

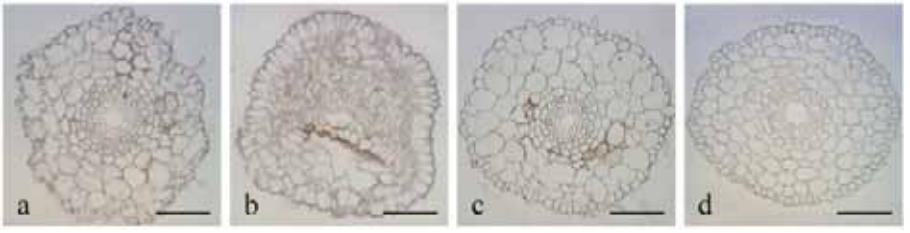

Figure 1. *In situ* localisation of *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* transcripts in 4-day nematode infected and uninfected resistant H-93-8 wheat root sections: A, *TaPrx111-A*; B, *TaPrx112-D* and; C, *TaPrx113-F* antisense probes. D, an example of uninfected root section which resulted not reactive to every probe antisense used. Scale bar indicates 2.8 μm in all figures.

Chromosome Mapping of *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* Peroxidase Genes

PCR amplification experiments by using genome-specific primers enabled the mapping of *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* peroxidase genes on the short arm of chromosome 2B as it can be deduced from lack of bands for nulli-tetrasomic N2B-T2D and ditelosomic DT2BL (Figure 2).

DISCUSSION

All *in situ* probes for the investigated peroxidase genes gave a strong reaction in cells injured by nematode migration in the resistant roots. This suggests that *TaPrx111-A*, *TaPrx112-D* and *TaPrx113-F* genes are regulated by wounding. Interestingly, infected roots from resistant wheat line showed heavy reaction in cells close to the vascular cylinder and parenchyma vascular cells when probed with

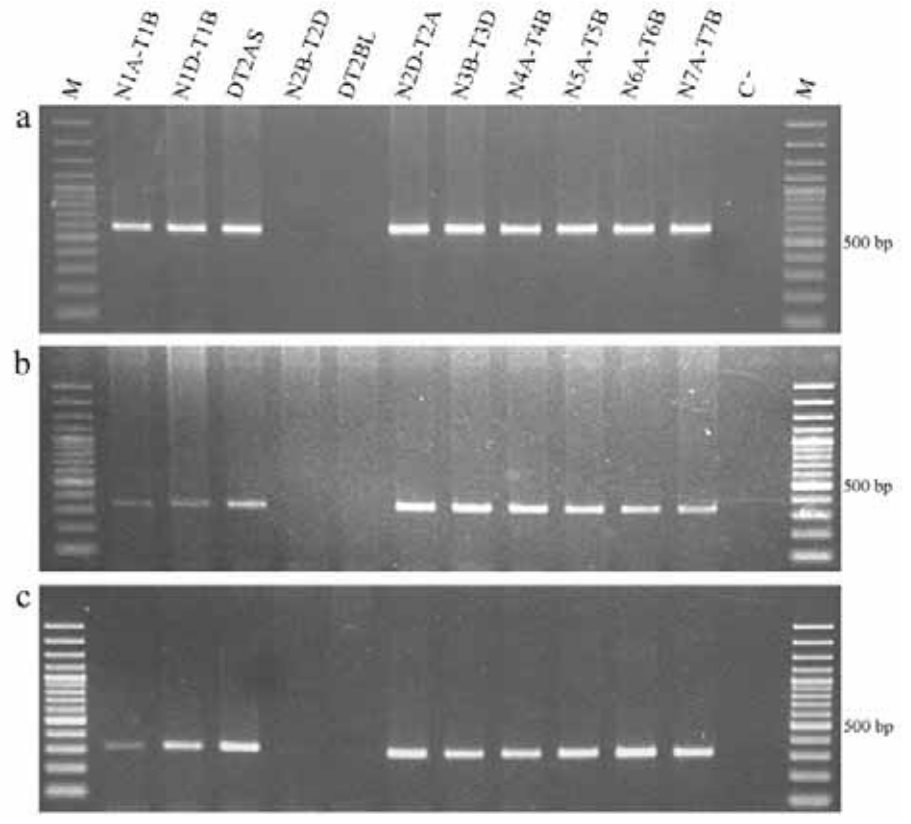

Figure 2. Electrophoretic analysis of PCR products obtained with specific primers (Table 1) for genes: A, *TaPrx111-A*; B, *TaPrx112-D* and; C, *TaPrx113-F* using genomic DNA from aneuploid wheat lines.

TaPrx112-D and *TaPrx113-F*. These peroxidases might, then, also play a role in the early resistance response to nematode injury.

Based on isoelectric point (pI) predictions mature proteins represented either acid TaPrx111-A, TaPrx113-F or basic TaPrx112-D isoforms of peroxidases, with pIs ranging from 5.78, 5.79 to 8.82. In a previous work Liu *et al.* (1990) identified five sets of genes encoding peroxidase isozymes by isoelectric focusing (IEF) of extracts from different tissues of hexaploid wheat cv. Chinese Spring. The *Per-2* group, carried on the short arms of group 2 chromosomes, showed the highest activity in root tissues with stained isozymes focusing around pH 9.0. Nullisomic analysis of root extracts, demonstrated that two of the Per-2 isozymes (designated as 3 and 4), were controlled by a gene(s) on the short arm of chromosome 2B. This leads to speculate that one of these peroxidases would match with *TaPrx112-D* gene product (pI 8.82), taking into account its overlapping expression pattern, pI and chromosomal location.

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NEMATODE RESISTANCE IN WHEAT TRANSGENICS EXPRESSING POTATO PROTEINASE INHIBITOR (*PIN2*) GENE AND ITS INFLUENCE ON PLANT YIELD AND NEMATODE FECUNDITY*

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SUMMARY

Cyst nematodes are obligate endoparasites of major crop plants. Of the various strategies used to combat these nematodes, transgenic plants with proteinase inhibitors as nematode anti-feedants is an important one. We have engineered a plant serine proteinase inhibitor (*Pin2*) gene into *Triticum durum* cv. PDW215 by *Agrobacterium*-mediated transformation to combat cereal cyst nematode (CCN, *Heterodera avenae*) infestation. The *pin2* systemic expression confers satisfactory nematode resistance and the proteinase inhibitor (PI) values show a direct positive correlation to plant height, plant seed weight and seed number. When the T_3 transgenic progeny lines were challenged with juveniles of *H. avenae* under controlled conditions, the transgenic lines showed an increase in plant height, photosynthetic yield, seed number and seed weight proportional to the proteinase inhibitor. A simultaneous decrease in nematode fecundity, cyst egg content and cyst number was observed, indicating that the PI was adversely affecting nematode infectivity and its growth in the host was seriously compromised. These results provide evidence that *pin2* gene from potato in wheat may represent a new source of genetic resistance against CCN available to wheat breeders.

*Khurana P, Chhibbar AK, Sashi V, Umarao (2009) Nematode resistance in wheat transgenics expressing potato proteinase inhibitor gene (*pin2*) and its influence on plant yield and nematode fecundity. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 200-203. (CIMMYT: Ankara, Turkey)

INTRODUCTION

Plant parasitic nematodes are major agricultural pests causing severe damage and economic losses worldwide in major crop plants like wheat and rice. The most important plant parasitic nematode in India is the cereal cyst nematode (*Heterodera avenae*), which causes severe damage to cereal crops such as barley and wheat, resulting in heavy economic losses. The application of nematicides, however, has been abandoned in many countries because of associated toxicity problems and environmental risks to the producer and consumer (Jung *et al*. 1998). Thus alternative molecular strategies for establishing nematode resistance in plant species have been pursued. Transgenic resistance can be designed to act either directly against the nematode or indirectly by disrupting the modified plant cells to which female cyst nematodes become developmentally committed (Atkinson, 1995). This has been achieved by introducing effector genes into the host plant that have a nematicidal impact. Cysteine proteinases are involved in protein metabolism and digestion of dietary protein, and shown to be present in nematodes. They are known to be inhibited by cystatins, which are small proteinase inhibitors (PI) and expression of cystatin in the transgenic plants prevents female nematodes from developing properly resulting in reduced size and fecundity in tomato hairy roots and *Arabidopsis* (Jung *et al*. 1998). Serine PI are the most extensively characterised class of plant PI that function in defence against herbivores (Lawrence and Koundal 2002).

In our previous study, a plant serine proteinase inhibitor gene (*Pin2)* from potato was introduced into wheat, and several transgenic lines showed enhanced growth and improved productivity (Vishnudasan *et al*. 2005). In order to assess the performance of transgenics showing stable expression of *Pin2*, we characterised the T_3 progeny of the previously raised *Pin2* lines with respect to plant yield and nematode fecundity under greenhouse conditions. Transgenic lines were challenged with the nematodes under controlled greenhouse conditions and allowed to develop till maturity maintaining an otherwise stress free environment throughout.

METHODS

Mature embryos of *Triticum durum* cv. PDW215 were transformed via *Agrobacterium*-mediated transformation with pCAMBIA:*Pin2* according to Patnaik *et al*. (2005). Screening of the putative transgenics was undertaken by PCR analysis for the genes, *Gus, Bar* and *Pin2*. Southern analysis was done to confirm the successful integration of the T-DNA in putative transformants. Expression of transgenes was quantified by reverse transcription PCR (RT-PCR) performed using SuperScript™ One-Step RT-PCR for long templates according to manufacturer's instructions (Invitrogen, Life Technologies). Selected transgenic lines were screened for resistance using standard bioassay methods described by Fisher (1982). Six transgenic wheat lines along with a non-transgenic control and a resistant variety Raj MR1 were screened for nematode resistance by inoculating with the juveniles of *H. avenae* biotype 1. Penetration studies were conducted to know the extent of penetration and establishment of *H. avenae* juveniles in different transgenic lines compared to the non transgenic control plants and a known resistant variety Raj MR1. The final cyst populations built up in the plants grown till maturity in the pots were calculated to estimate multiplication and nematode fecundity. Transformants were also screened for selected morphological and physiological parameters.

RESULTS

A total of 13 independent transgenic lines were generated by *Agrobacterium*-mediated transformation experiments for the introduction of *Pin2* for nematode resistance in *T. durum* cv. PDW215, via *Agrobacterium*–mediated mature embryo co-cultivation (Vishnudasan *et al*. 2005). *Bar* segregation and expression was evaluated by chlorophenol red assay suggesting stable integration, segregation as well as expression of the selectable markers in both T1 as well as T2 lines. Based on Southern analysis, *Agrobacterium*-mediated gene transfer efficiency was 3%, while the frequency of escapes was 36%. Apart from T0, T1 and T2 progeny analysis, six T3 transgenic lines were selected on the basis of RT-PCR analysis for further characterisation and biotic assay.

Roots were observed to be thinner and non-uniformly elongated in the challenged controls and transgenic lines as compared to unchallenged lines. Under challenged conditions, height of nearly all the transgenic lines was increased compared to the resistant line, Raj MR1, known to have a dominant gene for nematode resistance (Sharma and Sharma 2000). It was observed that irrespective of nematode infestation, the transgenic lines had a better photosynthetic yield in the form of F_V:F_M ratio as compared to the non-transgenics and were nearly equal in certain lines to the resistant line (Raj MR1). The PI values were observed to correlate significantly to plant height and, root and shoot growth indicating a direct influence of PI in conferring nematode resistance in the progeny lines. At harvest, seed number and weight were greater for infested transgenic lines compared to the uninfested lines.

The nematode establishment ranged from 42-62% across the six transgenic lines studied compared to about 42% in the non-transgenic lines. This indicates that penetration and development of the nematode was not affected by the protease inhibitor gene used in the transgenic lines. Final cyst number and egg content are two important parameters as they directly related to the overall growth of the plant. Compared to the control, number of eggs and juveniles were reduced in all the transgenic lines, except one line that had a lower PI content. Multiplication rate (final population/initial population) is an important parameter indicating nematode viability and fecundity. The presence of *Pin2* had a significant effect on nematode fecundity, as a result, nematode multiplication was much reduced in all the transgenic lines.

DISCUSSION

In this study, growth of CCN-infested transgenics was improved compared to the infested non-transgenic lines. However growth was better in uninfested transgenics. All lines differed significantly ($P<0.05$) from the non-transgenic lines with respect to eggs and juveniles per cyst, nematode establishment and photosynthetic yield. Our results thus provide evidence that in wheat, *Pin2* from potato represents a new source of genetic resistance to CCN available to wheat breeders. During the last decade there has been significant progress in the molecular characterisation of the interaction of cyst nematode and plants. The identification of both plant and nematode genes involved in key stages of parasitism will further aid the development of novel resistance strategies (Lilley *et al*. 2005). Also the use of nematode inducible plant genes will be valuable resources for creating new forms of durable plant resistance.

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PRELIMINARY FINDINGS OF TRANSGENIC APPROACHES TO MANAGE CEREAL CYST NEMATODE IN INDIA*

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SUMMARY

Among several biotic and abiotic stresses that limit wheat yields in India, cereal cyst nematode (CCN, *Heterodera avenae*) is one of the major problems. Since, the first report of CCN in India in a limited area, the nematode has been found in all wheat-growing areas of northern India. Evidence through host differential reactions and molecular genetic analysis of rDNA genes in recent years demonstrated considerable genetic diversity in its virulence and adaptability in various agroclimatic zones of India. *Heterodera filipjevi,* a species of the CCN complex, is reported to occur extensively. Therefore, national programs for resistance breeding have only identified a single resistance source. This, along with the lack of suitable chemical and agronomic control, necessitated research on understanding *H. avenae* genomics. Comparative genomics approach was employed to identify gene targets in CCN. Based on the information available on oesophageal gland genes in *Heterodera glycines*, two gland genes have been cloned and characterised from *H. avenae*. Additionally, six genes involved in different stages of feeding and development were identified and cloned using cDNA libraries of feeding females. Functional validation by RNA interference following *in vitro* feeding of dsRNA of respective genes has indicated encouraging results to employ these gene targets for transgenic wheat development. Our group has also launched a program to obtain the whole genomic sequence and the transcriptome of Indian CCN to better understand the compatible CCN-wheat interaction to allow design of more efficient management tools.

INTRODUCTION

India produces around 75 Mt of wheat, representing 12% of world production, and is the second largest producer of wheat in the world, next only to China. It is the major staple food grain in India after rice. Wheat accounts for 35% of the total food grain

*Umarao, Sharma A, Rao SB (2009) Preliminary findings of transgenic approaches to manage cereal cyst nematode in India. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 204-207. (CIMMYT: Ankara, Turkey)

production in the country and 37% of total cereal output. However, wheat productivity has been stagnant at an average 2.7 t/ha for the past six years. Several biotic and abiotic factors limit wheat productivity in the country. Cereal cyst nematode (CCN) is one of the major problems not only for wheat but also of barley, oat, rye, maize and other poaceous plants.

Heterodera avenae was first detected long before the green revolution on roots of wheat from the village of Nimka Thana, Sikar District, Rajasthan State (Vasudeva 1958). During the last 51 years, CCN has gradually been found more widely in India and now infest some 0.15 Mha in Rajasthan, and also being found in neighbouring states. Currently, it has been recognised as an endemic problem in the agroclimatic zones of northern to central India comprising the states of Punjab, Haryana, Uttra Pradesh, Himachal Pradesh, Jammu and Kashmir, Delhi and Madhya Pradesh.

YIELD LOSS

There are several studies reporting yield losses in farmer's fields. It was observed that CCN could cause about 40 to 50% yield loss reaching up to 60 to 65% (Mathur *et al.* 1980). In another study based on response to nematicide treatment, average avoidable losses ranging from 32 to 44% in barley and 24 to 35% in wheat have been reported (Handa and Yadav 1991). Of the 6.2 Mt of wheat produced in Rajasthan, the CCN infested area produced only 0.2 Mt in 2003-2004 instead of yielding 0.5 Mt, representing a loss of US$3.89 million (Sharma *et al*. 2007).

VARIABILITY OF CEREAL CYST NEMATODE IN INDIA

Earlier work in India to determine variability in *H. avenae* using international differentials including clones of a few grasses, revealed the occurrence of 5 biotypes in the state of Rajasthan alone (Mathur *et al.* 1974). Later, two biotypes have been reported in another study by Swarup *et al.* 1979. Biotype I comprised populations from the states of Rajasthan (Jaipur and Udaipur) and Haryana (Narnaul). On the other hand, biotype II included populations from the state of Punjab (Hoshiarpur and Ludhiana districts). An All India Coordinated Research Project carried out screening of wheat germplasm for resistance to CCN at multiple locations to identify sources for breeding and observed a large variation in the reaction of the germplasm which could be attributed to the variability in the parasite.

Our laboratory conducted initial studies on genetic variation among seven Indian CCN populations from major wheat growing states using RAPD–PCR Cluster analysis (Umarao *et al.* 2007). Using 148 scorable markers generated by nine random primers, seven populations were grouped into one cluster and two out groups; populations from major wheat growing locations Sirsa, Tikamgarh, Udaipur and Jaipur formed one cluster, whereas, each of the populations from Delhi and Jhansi formed two independent out groups. Further characterisation of these populations using DNA sequence variation in the non-coding regions of ribosomal DNA indicated more intraspecific variation (Umarao *et al.* 2004). Later studies based on host differential reaction, isozyme profile and molecular characterisation of Indian CCN biotype II population revealed that it is *Heterodera filipjevi*, a subspecies in the *H. avenae* species complex (Bishnoi and Bajaj 2004, Bishnoi *et al.* 2004, Umarao and Sashi 2008).

GENOMICS FOR DEVELOPMENT OF CERAL CYST NEMATODE RESISTANT WHEAT

In view of the limitations of the current management approaches, novel tools and techniques are needed to manage these nematodes. New approaches could be developed following the elucidation of the complete genome sequence or whole transcriptome sequence of *H. avenae*. Comparative genomics using information available for *Caenorhabditis elegans*, *C. briggsae*, *Meloidogyne hapla, M. incognita, Heterodera glycines* and *Brugia malayi* will provide insight into evolution of both parasitic ability and general nematode development. In addition there are about 530,000 ESTs available for different nematodes including plant, animal and human parasites. The comparative genomics studies may reveal critical junctures in the life cycle of *H. avenae* that may be unique and specific targets for anti-nematode therapies. In particular, events such as sex determination, arrested development and response to host and environmental cues may be examined in a detail previously impossible. These events represent breaking points in the nematode's life cycle, even though the mechanisms will be conserved between species, although the precise machinery may differ.

Presently there is limited information on the CCN genes involved in parasitism. Preliminary studies were undertaken in our laboratory to identify some important gene targets in CCN through *in silico* analysis, comparative genomics of existing EST and genome database of other cyst nematodes like *Heterodera schachtii, H. glycines* and *Globodera pallida*. As a result, we have identified, cloned and sequenced genes having role in CCN development, feeding and reproduction. Based on the information available on oesophageal gland genes of *H. glycines*, partial sequences of two genes (about 300 bp) were obtained by amplifying first strand cDNA prepared from young, feeding females of *H. avenae* and fuller lengths were obtained by screening a cDNA library. Additionally, six more genes involved in different stages of feeding and development were obtained by probing the library with selected conserved sequences from *H. schachtii, H. glycines* and *G. pallida*. The genes of *H. avenae* identified by the above procedures had homology to endoglucase, ubiquitin-like proteins, serine and cysteine proteases and RanBPM secretions. A full length sequence (1050 bp) has been obtained that may be a homologue of *syv46* in *H. glycines*. The gene in *H. avenae* contains seven repeats of a 29 amino acid sequence and, importantly, a conserved 13-aa C-terminal motif characteristic of the plant CLE peptide family. Interestingly, the homologue from *H. glycines* is known to rescue a developmental mutant of *Arabidopsis* that lacks a member of this plant gene family. *In vitro* silencing of this gene using standard protocols of RNAi approach for feeding the invasive juveniles with double stranded RNA gave about 37% reduction in both the number of cysts produced per plant and the eggs per cyst. Similarly functional validation by RNA interference following *in vitro* feeding of dsRNA of other genes has been done. Silencing of dorsal oesophageal gland gene gave about 76% reduction in the number of cysts produced per plant and 46% reduction in number of eggs per cyst when compared to control. Likewise silencing of other genes also has resulted in reduction of both number of cysts produced per plant and number of eggs per cyst. Work is in progress for *in planta* expression of dsRNA of these genes so as to deliver/introduce the dsRNA through the plant into the feeding juveniles to silence the genes.

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CLONING OF PARASITISM GENES FROM THE MEDITERRANEAN CEREAL CYST NEMATODE, *HETERODERA LATIPONS*[*]

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SUMMARY

The Mediterranean cereal cyst nematode, *Heterodera latipons,* attacks barley grown in the Northern and Southern Mediterranean phytogeographical zones and Eastern Desert of Jordan with variation in incidence and severity. The aim of this study was to clone a candidate parasitism gene from local populations of *H. latipons.* These targeted genes encode secretory protein products. Three populations of *H. latipons* from Ar-Ramtha, Madaba and Dana were identified. Morphological studies were used for all populations to confirm species identity before any molecular work. Pectate lyase and chorismate genes were the target genes. Therefore primers were designed based on alignment from *H. latipons*. Certain primer combinations showed amplicons using genomic DNA (directly from second-stage juveniles). A total of 35 amplicons were cloned and 18 of them were sent for sequencing. These clones were analysed using Blastx searches against sequences in NCBI databases. Blastx resulted in similarities to putative proteins such as digestive gland protein, glutamyl transpeptidedase and putative ubiquitin. The open reading frame of ubiquitin extension protein was 300 bp long. The cloned ubiquitin sequence from *H. latipons* has a high similarity to ubiquitin from *Heterodera schachtii* and *H. glycines*.

INTRODUCTION

In Jordan, field surveys revealed that Mediterranean cereal cyst nematode occurred in Northern and Southern Mediterranean zones and Eastern Desert (Al Abed *et al*. 2004). Incidence and severity studies showed that these nematodes were dominant and severe in Ar-Ramtha and Mafraq, moderate in Al-Karak and not found in newly

[*]Lafi HA, Sadder M, Al-Banna L (2009) Cloning of parasitism genes from the Mediterranean cereal cyst nematode, *Heterodera latipons*. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 208-212. (CIMMYT: Ankara, Turkey)

developed areas such as the southern region at Al Mudawarra (Al Abed *et al*. 2004). Study of virulence revealed variation between three geographical populations of this cereal cyst nematode (Al Abed *et al*. 2004). To understand such variation in the virulence, it is necessary to identify and characterise the parasitism genes, and to reveal the factors that make this nematode a parasite and cause of severe crop damage.

The mode of parasitism of this cysts nematode is mostly understood. It is well known that the penetration and the invasion of cyst nematodes and the formation of syncitia, the feeding site, are results of nematode esophageal secretions injected into the root recipient cells by a protrusible stylet. These secretions are encoded by parasitism genes. Examples of these parasitism genes are those which encode ubiquitin extension protein, enzymatic activity protein chorismate mutase and the cell wall modifying genes that encode cellulose binding proteins, ß-1,4-endoglucanase, and pectate lyases (Vanholme *et al*. 2004).

To date information has been published about the parasitism genes of the Mediterranean cereal cyst nematode, *H. latipons,* that is a serious pest of barley in Jordan. Therefore, this study was conducted to detect parasitism genes secreted by this cyst nematode.

METHODS

Populations of the Mediterranean cereal cyst nematode *H. latipons* were sampled from three different Jordanian barley geographic regions. Soil samples were taken from each region and were air dried.

Extraction of cysts was achieved by flotation (Shepherd 1970). Eggs were obtained by crushing the extracted cysts. Subsequently, second stage juveniles (J2) were obtained by allowing eggs to hatch at 10C. Extracted stages of the nematodes were mounted for identification or stored as a pellet at -80C for molecular studies.

Both qualitative and quantitative morphological characteristics of J2 and cysts were tabulated and used to identify the species following the original descriptions and the diagnostic keys (Schmidt 1871, Wollenweber 1924, Franklin 1969).

Cysts, eggs, J2 stored at -80C were thawed at room temperature for direct polymerase chain reaction (PCR). Several primers were designed with Vector NTI software (Invitrogen). The nucleotide sequences of pectate lyase and chorismate mutase of root knot and cyst nematodes were aligned using Vector NTI and thereafter four pairs of forward and reverse primers were designed from the conserved nucleic regions of the alignment.

Several screenings were performed on genome DNA (directly J2) with different pectate lyase and chorismate mutase primers combinations. All PCR products were run on gels and selected bands were gel purified using ENZA Gel Extraction Kit (200 N 2501-02, Omega Bio-tek, USA). For conformation of PCR, an actin and a rDNA region were used as positive controls. Actin primers were designed from the cyst nematodes sequences alignment. Whereas, universal primers D3A and D3B from *C. elegans* were used to amplify the D3 domain of 26S rDNA region (Al-Banna *et al*. 1997).

Amplifications were carried out for 35 reaction cycles. Each reaction was performed in 10 µl containing 2-3 nematodes in 1 µl distilled water were mixed with 1x reaction buffer, 200 µM deoxynucleoside triphosphate (dNTPs) (Promega, USA), 0.4 µM of each primer and 1 U of *Taq* DNA polymerase (Promega, USA). The amplification was performed in Biorad PCR (My Cycler) machine as follows: hot start 94C for 2 min, 35 amplification cycle at 94C for 1 min, 30C depending on the combination of the primers used for 1 min, 72C for 1.5 min and a final extension step for 10 min at 72C. Products were analysed by electrophoresis using 5 µl of each PCR sample with a loading buffer (6x Loading buffer bromophenol blue) loaded onto a 1% agarose gel prepared in 1x TAE buffer.

A Hi-Lo DNA ladder marker (Minnesota-Molecular Inc., USA) was used to estimate the approximate molecular weight of the amplified products. Generated bands were screened and digitally photographed under UV light (Alphaimager™ 2200, Alpha Innotech, USA). The amplicons from template (DNA) of the interested parasitism gene was cloned into the bluescript vector (pBS II SK +). The plasmid DNA was extracted using Quick Clean 5M Miniprep Kit (Cat. No. L00193) and then resuspended in 50 µl SDW. The Plasmid DNA was electrophoresed on a 1% agarose gel and visualised under UV light (Alphaimager™ 2200, Alpha Innotech, USA). The plasmid containing the insert was used for sequencing.

Sequence analysis was performed based on the nucleotide termination following Sanger's method. The sequences represented parasitism genes were analysed and compared with other organism sequences (genomic DNA) listed in the gene bank. Comparative analysis of nematode parasitism was done with other organisms with Blastx (National Center for Biotechnology Information, Bethesda MD, USA).

RESULTS

A total of 24 sequences using combinations of primers were obtained. Sequence length varied from 174 to 1258 bp. The sequence identities ranged from 22 to 73% over 32 to 90 aa residues. Sequences analysis of these DNA indicated that they may encode gene products of various functions. Similarity results were found with Blastx using the Genbank database. Blastx searches of amplicon sequences against NCBI databases revealed homologies to some sequences such as putative oesophageal gland proteins, hypothetical protein, heat shock protein, putative gland protein, glutamyl transpeptidedase and ubiquitin (Table 1). The identified ubiquitin gene from our target nematode, *H. latipons,* showed a good similarity to *H. glycines, H. schachtii* and *C. elegans*

DISCUSSION

The designed primers were not able to amplify pectate lyase and chorismate mutase sequences from *H. latipons.* However, the primers amplified several parasitism genes and other genes. This method is similar to earlier work by Rosso *et al*. (1999), who designed primers from the conserved peptidic regions YVIVDWH and FVTEYGT located in the catalytic domain from *H. glycines Hg*-eng-1 and *Hg*-eng-2 (GenBank accessions AF006052 and AF006053, respectively) and *G. rostochiensis Gr*-eng-1 and *Gr*-eng-2 (GenBank accessions AF004523 and AF004716, respectively) to clones similar genes from *M. incognita*.

Table 1. Similarity results for Blastx analysis.

Sequence designation	Length (bp)	Highest match protein	Source
MTS-4 (12.11.07)	242	Putative gland protein G16H02 Hypothetical protein CBG15673 Hypothetical protein H14E04.2b	*Heterodera glycines* *Caenorhabditis briggsae* AF16 *Caenorhabditis elegans*
MTS-7 (12.11.07)	366	Heat shock protein hsp-90	*Heterodera glycines*
MTS-8 (12.11.07)	314	28S ribosomal RNA gene	*Heterodera glycines*
MTS-10 (12.11.07)	242	Putative gland protein G16H02	*Heterodera gl*ycines
MTS-3 (1.7.08)	238	Glutamyl transpeptidedase	*Heterodera glycines*
MTS-4 (1.7.08)	423	Putative gland protein G8H07	*Heterodera glycines*
MTS-3 (12.11.07)	300	Ubiquitin family member (ubq-1) Ubiquitin extension protein 2 Ubiquitin extension protein	*Caenorhabditis elegans* *Heterodera schachtii* *Heterodera glycines*
MTS-1 (12.11.07)	360	28S ribosomal RNA gene	*Heterodera latipons*

About 70% of the DNA and cDNA amplified bands were cloned and sent to sequencing by a commercial service. However, some of them did not produce readable sequences. The submitted plasmids may have degenerated with DNase during shipping or the clone may have had multiple inserts.

A Blastx search of the nucleotide sequences of these clones revealed that the majority of the aa sequences of the identified inserts had significant identity with putative proteins. One notable feature is the clone that encodes the ubiquitin extension protein. The results showed significant sequence identity with ubiquitin extension protein in cyst nematodes (65 to 73% sequence identity over 57 to 63 aa residues).

The identified ubiquitin gene from our target nematode, *H. latipons,* showed a good similarity to *H. glycines, H. schachtii* and *C. elegans*. The nematode ubiquitin gene products have significant similarity to plant genes and are involved in selective host cell protein degradation (Gao *et al*. 2003, Tytgat *et al*. 2004). The identify ubiquitin gene from both cyst nematodes, *H. glycines* and *H. schachtii,* are originated in the dorsal oesophageal gland (Gao *et al*. 2003, Tytgat *et al*. 2004). Furthermore, Gao *et al.* (2003) reported that their identified ubiquitin was expressed in the pre- and early parasitic juveniles of *H. schachtii* by performing cDNA AFLP on these developmental stages. Our results showed that the identified ubiquitin was cloned from genomic DNA from J2s of *H. latipons*. However, further work is needed to detect the expression of this gene from all developmental stages. Such work will be helpful for future studies on silencing ubiquitin or knocking down their expression using RNA interference, to avoid the formation of syncytium, so that the nematode will not grow further and result in suppression of damage to the host crop.

On the other hand, the remaining clones showed little to moderate sequence identities with other nematodes. The sequence identities ranged from 22 to 36% and over 32 to 90 aa residues. The heat shock proteins are any of a group of specific proteins that are synthesised by both prokaryotic and eukaryotic cells after they have been exposed to a higher than normal temperature. Other stresses, e.g. free radical damage, have a similar effect. Many members of the heat shock protein family are

not induced but are present in all cells. They are characterised by their role as molecular chaperones (Schlesinger 1990).

Glutamyl transpeptidase is involved in the transfer of amino acids across the cellular membrane. It is also involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water, certain L-amino acids and peptides leaving the cysteine product to preserve intracellular homeostasis of oxidative stress (Yokoyama 2007). Therefore, these cloned genes must be further investigated for their expression, sequence, hybridisation, and similarity to related species (phylogenic relationship).

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PART 5 MANAGEMENT BY OTHER APPROACHES AND INTEGRATION OF STRATEGIES

INCREASED BIODIVERSITY IN CEREAL CYST NEMATODE INFESTATIONS IS NOT A THREAT TO INTENSIVE CEREAL PRODUCTION IN SOUTHERN BRITAIN*

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SUMMARY

In a limited survey of cereal fields in southern Britain in 2006, 65% of the samples were infested with cereal cyst nematodes. Morphological, biochemical and host range studies showed that most fields contained mixed populations of *Heterodera avenae* and *H. filipjevi* and produced a single generation in a growing season. Nematode females and eggs were parasitised by fungi, including *Catenaria auxiliaris* and *Pochonia chlamydosporia,* in all populations studied and results indicated that these fungi limit reproduction of these nematodes on susceptible crops. The plant defence compound DIBOA (a hydroxamic acid produced by wheat and rye) reduced hatch and mobility of *H. avenae* juveniles and increased mortality; *H. filipjevi* juveniles recovered from exposure to this compound. *H. filipjevi* is not a significant new threat to UK cereal production and is currently controlled by those fungi that have also successfully limited *H. avenae* populations. The widespread distribution of *H. filipjevi* in the UK may have resulted from a change from spring to autumn cereal cropping.

INTRODUCTION

Cereal cyst nematodes (*Heterodera* spp.) are a major constraint to cereal production in many parts of the world. In the UK, nematode populations were reduced to levels below the economic damage threshold of 5 eggs/g soil under cereal monocultures (Gair *et al.* 1969), and two fungal antagonists, *Nematophthora gynophila* and *Pochonia chlamydosporia,* were found to be responsible for maintaining this low

*Mitchinson S, Gowen SR, Kerry BR (2009) Increased biodiversity in cereal cyst nematode infestations is not a threat to intensive cereal production in southern Britain. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 215-220. (CIMMYT: Ankara, Turkey)

level (Kerry 1982a,b). Recent studies in Norway showed that another species, *Heterodera filipjevi* was causing significant damage to cereals (Holgado 2003) and that soils in Norway may be conducive to this species. The 'suppressiveness' of a soil can be tested by the application of a partial soil sterilant (Kerry *et al*. 1980, 1982a, Westphal and Becker 1999). The present study aimed to characterise UK cereal cyst nematode populations and establish whether *H. filipjevi* is present and whether it is a potential new threat to cereal production. Soils were tested for their 'suppressiveness' and the role of wheat and rye plant defence compounds (hydroxamic acids) was investigated.

METHODS

A survey of 23 cereal fields in southern Britain was conducted in 2006. Field populations were characterised using a combination of morphological, biochemical (iso-electric focusing) and molecular (PCR-RFLP) techniques and by use of an international test assortment of barley and oat cultivars. An outdoor pot experiment using wheat and rye sown in field soils was designed to determine the number of generations UK populations have in a single growing season. Pots were sampled every four weeks and the roots and soil examined for nematode life stages. A further pot experiment in the glasshouse used formalin application of soil as a partial soil sterilant. Nematode populations were regularly sampled for the presence of females and cysts. Observation chambers containing field soil sown with wheat and barley were used to study female infection with fungi and length of time until female infection and disintegration. Hatch and mobility/mortality of *H. avenae* and *H. filipjevi* juveniles on exposure to varying concentrations of DIBOA (a hydroxamic acid) was studied *in vitro*. A further test was conducted to investigate whether the observed effects on juvenile mobility were reversible, by transferral of juveniles to water after 48 h exposure to DIBOA.

RESULTS

The survey of cereal fields in southern Britain revealed the presence of mixed *Heterodera* species, mainly *H. avenae* and *H. filipjevi*. Sixty five per cent of infested fields contained *H. filipjevi* suggesting that this species is more prevalent in the UK than previously thought. One generation of cereal cyst nematode was produced in a single growing season, as shown by a single peak in female production (Figure 1), indicating that UK populations of cereal cyst nematodes are monocyclic. The number of cysts decreased over sampling time suggesting that the populations were in decline.

Formalin application resulted in a slight nematicidal effect, which reduced numbers of females and cysts in treated soil. However, there was a decrease in the number of females that was not accounted for by an increase in cyst production (Figure 2), suggesting that the soil in the non-treated control pots was also suppressing nematode multiplication. A small percentage of eggs within mature cysts were infected with *Pochonia chlamydosporia*. Studies using observation chambers revealed the presence of *Catenaria auxiliaris* spores in infected females removed for microscopic analysis, with almost 100% mortality in all of the populations tested. Females were infected and disintegration occurred from 3 to 29 days after female emergence on the root.

Hatch of *H. filipjevi* juveniles was reduced when cysts were exposed to the hydroxamic acid DIBOA. Mobility of juveniles was reduced in both *H. avenae* and *H. filipjevi* juveniles after hatch, with LD50s (50% of maximum mortality) of 29 and 11µg/ml, respectively (Figures 3 and 4). Despite a lower LD50 for *H. filipjevi*, a maximum of 51% mortality was achieved and no further increase in mortality was found with concentrations of DIBOA above 40 µg/ml. For *H. avenae*, a higher mortality (57%) was achieved. The adverse effects of DIBOA on juvenile mobility were temporary and reversible for *H. filipjevi*, especially at high concentrations, but less so for *H. avenae* where mortality occurred (Figure 5).

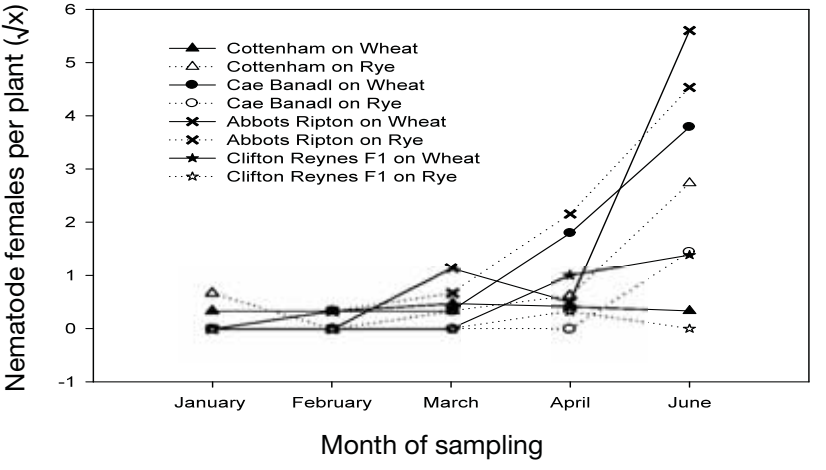

Figure 1. Square root mean number of females produced per pot for four field populations on wheat and rye.

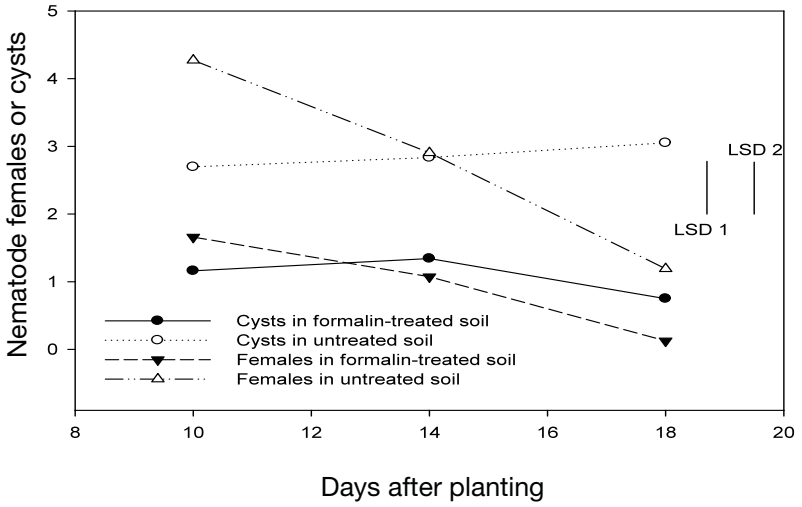

Figure 2. The number of cysts or females over time in formalin treated and untreated soil from Abbots Ripton.

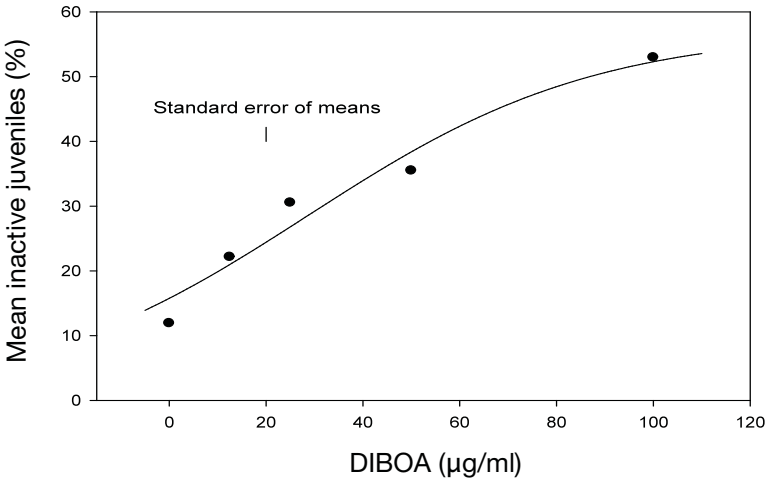

Figure 3. Mean per cent inactive juveniles for days 13-33 for each concentration of the hydroxamic acid DIBOA tested for *H. avenae.*

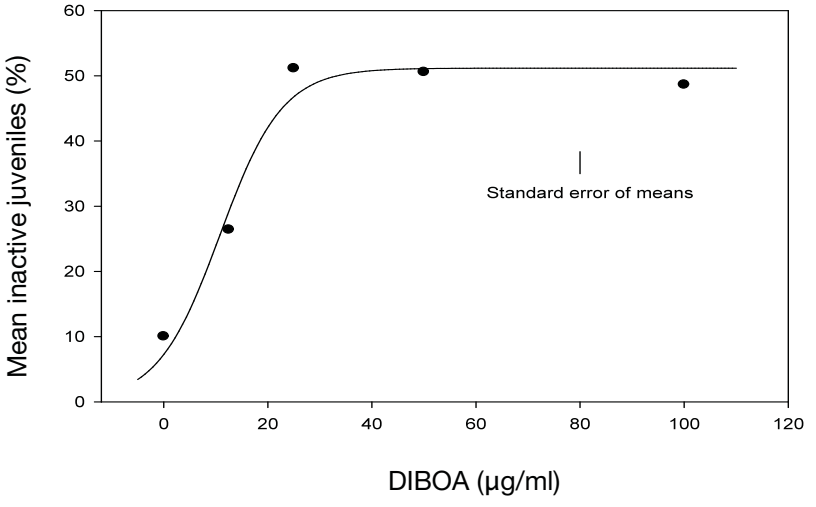

Figure 4. Mean per cent inactive juveniles for days 14-33 for each concentration of the hydroxamic acid DIBOA tested for *H. filipjevi*.

DISCUSSION

Heterodera filipjevi occurred in 65% of infested cereal fields studied in this survey and is more widespread than previously thought. This species has been found in many countries and has caused significant economic damage to wheat, especially under sub-moisture systems (Nicol *et al*. 2006). It is possible that in the UK this species was previously misidentified as *H. avenae,* as the two species are morphologically similar. However, changes in agronomical factors such as a switch

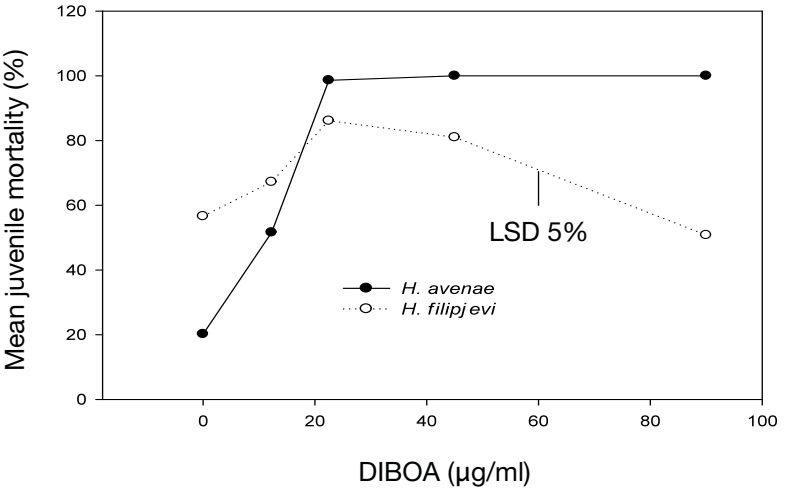

Figure 5. The effect of DIBOA concentration on per cent juvenile mortality for *H. avenae* and *H. filipjevi*, after transferral of juveniles to sterile distilled water.

from spring to autumn sown cereals may have increased the spread of this species in the UK.

The obligate parasite, *C. auxiliaris*, was found to parasitise almost 100% of females in observation chambers, in this study. This species of fungus also parasitised cyst nematode females, including *Globodera pallida*, *H. avenae* and *H. schachtii* (Tribe 1977). *Pochonia chlamydosporia* infected eggs but *N. gynophila* was not found in females in the observation chambers, despite being responsible for the suppressive soils that kept *H. avenae* populations below the economic damage threshold (Kerry 1982a,b).

The hydroxamic acid, DIBOA, reduced hatch of *H. filipjevi* juveniles, and reduced mobility and increased mortality of *H. avenae* and *H. filipjevi* juveniles. Nematode locomotion relies on the neurotransmitter, acetylcholine, which is deactivated by acetyl cholinesterase (AChe) (Opperman and Chang 1990). DIBOA may inhibit AChe in nematodes and cause temporary paralysis. Differences in species response to hydroxamic acids may be due to the amount of AChe that the nematode possesses (Al-Rehiayani 2008), with species containing higher amounts able to tolerate higher doses.

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CROP ROTATION AND *HETERODERA AVENAE* (CEREAL CYST NEMATODE) POPULATIONS IN SPRING CEREALS AT HIGH ALTITUDE IN QINGHAI, CHINA*

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SUMMARY

Cereal cyst nematode (*Heterodera avenae*) population densities were determined in spring cereals at harvest in two high-altitude villages in Qinghai, China in order to examine the effect of crop rotations. The two rotational systems sampled were wheat with rapeseed, broad bean and/or potato, and barley with rapeseed and/or oat. The previous season's crop, including fields where two host crops had been grown in succession, did not appear to influence the final nematode density. A high degree of variation in population density appeared to be strongly influenced by the occurrence of hyperparasites, thus masking any possible crop rotation effects. Nevertheless, a third of the fields had final egg densities greater than 10 eggs/g soil, creating a risk of yield loss if an intolerant host was to be grown in the next year. From the findings, it is suggested that future research should focus on developing locally-adapted resistant cultivars and examining factors that determine the efficacy of natural biocontrol.

INTRODUCTION

In China *Heterodera avenae* was first recorded in Hubei about 20 years ago (Chen *et al.* 1989). Subsequently, CCN was shown to be widespread in Hubei (Wang *et al.* 1991), as well as in Henan and Hebei (the two major wheat growing provinces), as well as in Shanxi and the rural areas of Beijing (Chen *et al.* 1992). Interest in CCN and its impact in China has increased in recent years (Peng *et al.* 2007, Riley *et al.*

*Riley IT, Hou SY, Chen SL (2009) Crop rotation and *Heterodera avenae* (cereal cyst nematode) populations in spring cereals at high altitude in Qinghai, China. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 221-226. (CIMMYT: Ankara, Turkey)

2007). However, this work has focused on irrigated winter wheat, with some survey activity in spring wheat and barley in provinces such as Gansu, Inner Mongolia and Qinghai (Peng *et al.* 2008).

The goal of this study was to investigate the role of crop rotation on variation in CCN population density in spring wheat grown at high altitude in Qinghai. Knowledge of the influence of crop rotation on CCN populations would be helpful to farmers needing to manage this pest until the resistance status of the common cultivars is determined or new locally-adapted resistant cultivars become available.

METHODS

Thirty five fields were sampled in a village in Huang Zhong county (alt. 2,570 m) where spring wheat (*Triticum aestivum* subsp. *aestivum*) is grown in rotation with rapeseed (*Brassica napus*), broad bean (*Vicia faba*) and/or potato (*Solanum tuberosa*) (Table 1). Rotations were mostly one year broadleaf crop followed by one year of wheat. Twelve fields were sampled in a village in Huang Yuan, a higher altitude village (alt. 3,100 m) with a growing season too short for wheat. Consequently, barley (*Hordeum vulgare* subsp. *vulgare* convar. *vulgare* var. *trifurcatum*) is grown in rotation with oats (*Avena sativa*) and/or rapeseed, giving two common rotations, rapeseed-oats-barley and rapeseed-barley (Table 1). These villages were selected as representative of the common agricultural practices in the areas known to be infested with *H. avenae*. Fields in Huang Zhong were terraced, bunded and flood irrigated and in Huang Yuan they were rain-fed (Figure 1).

Table 1. Crop rotations in fields sampled in two counties (one village each) in spring cereal growing areas, Qinghai, China.

County	Crop 2008	Crop 2007	Fields sampled	Crop 2006	Crop 2005
Huang Zhong	Wheat	Wheat	4	Rapeseed	Wheat (1 rapeseed/ potato split)
		Rapeseed	8	Wheat	Mostly broad bean, rapeseed or potato (or split with potato; 2 wheat)
		Broad bean	13	Wheat	
		Potato	6	Wheat (1 rapeseed)	
		Rapeseed/ potato split	2	Wheat	
		Broad bean/ potato split	2	Wheat	
Huang Yuan	Barley	Rapeseed	8	Barley (1 Oats)	Rapeseed
	Oats	Barley	4	Rapeseed	Oats (1 barley)

Fields that had grown either wheat, barley or oats were sampled (1-12 September 2008), most crops having been hand or machine harvested, but none had been cultivated. Forty five cores (100 mm deep by 10 mm diameter) of top soil were collected in a grid pattern across the entire field and combined. Information on the crops grown in the preceding three years (2005-2007) was provided by farmers.

Figure 1. Fields at harvest in Huang Zhong (A) and Huang Yuan (B), Qinghai.

The samples were air dried and processed in entirety to avoid subsampling errors. Organic matter between 250 and 720 µm was collected by wet sieving and again air dried for later processing. The cysts from the dry organic matter were extracted by flotation and wet sieving (250 µm) and collected on fluted filter paper in a conical funnel. All apparently full cysts were collected during examination of the sample under a stereo microscope. Empty and full cysts in the sample were visually ranked on a 5 point scale; 0 = no cysts, 1 = 1 to 50 cysts per sample, and 4 = >500 cysts per sample, with 2 and 3 being the approximate lower and upper ranges between 1 and 4. Ranks were calibrated by counting all cysts in representative samples. This rank is considered an indicator of the history of infestation, as old cysts persist in the soil for many years in this environment. Observations on hyperparasitism and cyst size were also recorded. Apparently full cysts were re-examined (25**x**) and any that were found to be empty or parasitised were discarded. All cysts, or a subsample of 10, were crushed, suspended in 10 ml of water and eggs counted in three 1-ml aliqouts.

RESULTS

All fields were infested with *H. avenae* as evidenced by the presence of characteristic cysts. Egg densities non-normally distributed between 0 and 70 eggs/g soil (Figure 2a), with high densities only found in Huang Zhong. Cysts with eggs were not found in 7 fields, but infestation was evidenced by the presence of empty cysts. The frequency of cyst rankings are shown in Figure 2b. Cyst ranks in Huang Yuan were significantly greater than Huang Zhong ($P<0.001$, $U=60$, Mann-Whitney U test). The number in the most heavily infested sample was estimated at 827 cysts (1.4 cysts/g soil).

The 2008 crop, wheat (Huang Zhong), barley and oats (Huang Yuan), did not significantly effect the egg population densities measured; neither did the 2007 crop, even when this was a host of *H. avenae*.

A moderate to high degree of hyperparasitism was evident as either (1) brown eggs, J2s undeveloped and clumps of eggs not readily dispersed in water, or (2) eggs being replaced entirely with smaller, more spherical oomycete structures, mostly likely to be zoosporagania, and hyphae. The relationship between total cysts and egg densities was tested and showed a significant quadratic effect ($P=0.02$), consistent with high ranks being associated with decline in egg density (Figure 3a). Obvious parasitism of cysts in high ranked samples was also associated with large number of

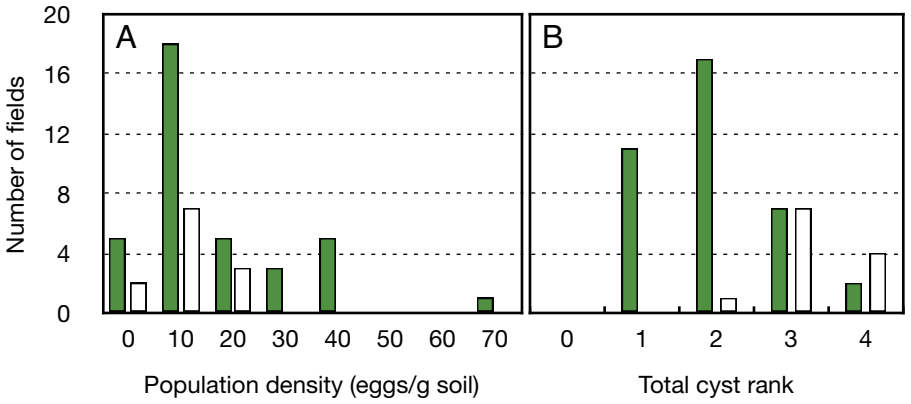

Figure 2. Frequency of population densities (A) and total cysts (B) of *Heterodera avenae* found in 49 infested fields in two villages (Huang Zhong, filled; Huang Yuan, unfilled) in Qinghai, China. Total cysts extracted from the soil samples (c. 500 g) were visually ranked on a 0-4 scale, where 0 = no cysts, 1 = 1 to 50 cysts, and 4 = > 500 cysts, with 2 and 3 being lower and upper densities between these limits.

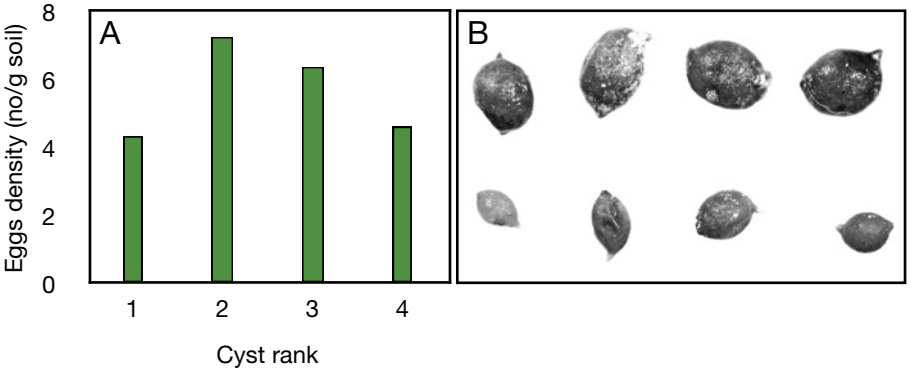

Figure 3. A. Relationship between total cyst ranking (ranks 1-4 on 0-4 scale) and egg densities of *Heterodera avenae* in fields in Huang Zhong, Qinghai, China. Quadratic fit (P=0.02); individual densities are not significantly different. B. Normal (upper) and parasitised (lower) cysts of *H.* avenae common in Huang Yuan, Qinghai, China.

small empty cysts, which would occur if *H. avenae* females are parasitised before maturity (Figure 3b). In Huang Yuan, egg densities were lower and cyst ranks higher than in Huang Zhong (P=0.05; Figure 2). Consistent with this, samples from Huang Yuan contained many parasitised cysts and a high proportion of small empty cysts, as illustrated in Figure 3b.

In addition to CCN, low numbers of mostly empty cysts of *Cactodera thornei,* previously recorded in Qinghai (Peng and Vovlas 1994), were found in five fields in Huang Yuan, but the host in these sites is not known.

DISCUSSION

A high frequency of CCN infestation in fields of two villages in the long-established cropping areas in Qinghai was found. Although the frequency of infestation was high, there was a wide range in population densities. At the low end of the range, infestation was evidenced by empty or parasitised cysts only, with cysts potentially containing viable eggs presumably at a density below the detection limit of the methods employed. However, a third of the fields had over 10 eggs/g soil indicating considerable potential for yield loss, if intolerant host crops were grown in the next year. The economic threshold for CCN in spring wheat at sowing can be as low as 1 egg/g soil (Andersson 1982), so even after an intervening non-host crop, yield loss might occur, assuming CCN attrition rates are consistent with the 70-85% observed in other countries (Stanton and Fisher 1979, Andersson 1982).

Although the predominant crop rotation practised is one year of CCN host (wheat or barley) followed by one year of non-host (broad bean, rapeseed or potato), there were insufficient fields sampled with two host crops in sequence (wheat-wheat or barley-oats) to determine if this would lead to elevated CCN densities. There was no evidence that a second host crop increased CCN densities and no relationship was found between the other rotational crops and CCN densities. So although rapeseed might provide some biofumigation effects against nematodes (Halbrendt 1996), there was no evidence that it or any of the other rotational crops substantially lowered CCN densities. These findings indicate that other factors are likely to be driving CCN densities, potentially masking any crop rotation effects.

If the variation in CCN population densities is not determined by rotational practices, it appears from our data and observations that natural biological control is most likely. The considerable number of parasitised cysts and small cysts observed, and the disparities between total cysts and egg counts, points to microbial antagonism being an important determinant of CCN multiplication and survival rates in these soils. Hyperparasitism of CCN and other heteroderid species is well known (Kerry 1980, Kerry and Crump 1989) and is best developed in moist soils with a high frequency of host crops. In contrast, southern Australia's relatively dry wheatbelt soils, parasitism of CCN cysts is infrequent (~1%; Stirling and Kerry 1983). Conditions in Qinghai appear conducive to the development of of natural biocontrol of CCN.

In conclusion, this study has shown high frequency of CCN infestation and variability in population densities in two cropping areas of Qinghai, and highlighted a potential for crop losses (based on densities observed). Significant variation in population density appeared to be driven by hyperparasitism, but more needs to be learnt about the organisms and processes involved. Given that CCN in other countries can be effectively controlled by host resistance, and assuming yield losses in Qinghai are confirmed, it is recommended that future research focus on breeding locally-adapted cultivars with appropriate resistance, and developing an understanding of natural suppression and how it might be manipulated for favourable outcomes.

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THE EFFECT OF ROOT EXUDATES FROM CERTAIN EGYPTIAN MEDICINAL PLANTS ON THE CYST NEMATODE, *HETERODERA ZEAE*[*]

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SUMMARY

Cyst nematodes exist in certain locations in Egypt, but its economic importance is considered less than that of root knot nematode because of the wide host range of *Meloidogyne* species. Various studies showed the presence of nine species of *Heterodera,* mostly in clover, corn, peanut, rice, cotton, cowpea and wheat. Seven plant species used for medicinal purposes in Egypt were tested in microplots and in small field plots to determine their effects on cyst nematode, *Heterodera zeae.* Five of these, *Ambrosia maritima, Calendula officinalis*, *Hyoscyamus muticus*, *Linum usitatissimum*, and *Origanum vulgare*, significantly reduced the number of nematodes when planted into microplots inoculated with *H. zeae* or in naturally infested field plots. Root exudates from these plants were collected and tested *in vitro* to observe their effect on hatch and/or mortality of *H. zeae*. Hatch increased in the presence of *C. officinalis, H. muticus* and *L. usitatissimum* when compared to the controls, whereas a lower rate of hatch occurred with exudates of *A. maritima* and *O. vulgare.* Survival of hatched juveniles was significantly reduced after 24 h in exudates of *A. maritima, C. officinalis* and *O. vulgare* when compared to the controls. Juveniles, inoculated onto root explant cultures with added exudates, failed to penetrate and/or develop. These results indicated that some medicinal plants may be effective as natural nematicides in the control of this nematode.

INTRODUCTION

Plant disease caused by different species of cyst nematodes had been observed for many years. The first of this group of nematodes was recognised in 1881 in Japan as

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Heterodera schachtii, and subsequently in Korea 1936. In 1954, soybean cyst nematode was detected in United States and has continued to be studied since as a significant limiting factor in crop production.

In Egypt soybean cyst nematode was reported (Diab 1968) in cowpea in 1968 and later reports (Aboul-Eid *et al.* 1974) indicated that *Heterodera cajani* accounted for at least part of the cyst population in cowpea in Egypt. Oteifa reported (Riggs 1977) that SCN does occur in soybean on some island in the Nile River.

Pesticides have been used increasingly to control these nematodes, especially on high value crops. Recently most pesticides have come fallen under the scrutiny of the Environmental Protection Agency and some are listed as RPAR (Reputable Presumption against Registration). Accordingly, research efforts are directed towards the discovery of safe and selective methods for pest control lacking hazardous impact on the environment while limiting pest competition.

It was found out that plants use a variety of secondary metabolites to protect themselves from pest and disease. Among these defensive compounds, there are those with repellent and pesticides activity. The co-evolution of plants and pests has resulted a wide variety of chemical interaction that balance and permit their coexistence (Byrd *et al.* 1983). Among these chemical ecological interactions, it is expected to find new chemistry in plants which affect pest feeding or disrupt reproduction, life cycle and/or behaviour as natural and biologically safe methods for pest management.

Previous investigations approved that these natural products are composed of natural, stabilised plant and mineral extracts which when applied to the plant or soil in part biocidal properties within the plant and rhizosphere (Husain and Mahmoud 1975). Also, the natural compounds help conserve and upgrade the environmental conditions while improving crop production (Okada 1971).

A recent study was conducted in Egypt evaluated medicinal and ornamental plants as rotation crops. These high value crops in Egypt are normally used commercially for general medicinal purposes. The objective of this investigation was to determine if certain medicinal and ornamentals plants could be a provide a new means of nematode control. Extracts from these plants were tested *in vitro* to observe if natural nematicidal activities might result from these plant extracts.

METHODS

Plant Material

Foliage of *Ambrosia maritima*, *Calendula officinalis, Hyoscyamus muticus* and *Origanum majorana* (Figure 1), and the seeds of *Linum usitatissimum* were collected from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University.

Root Explant Cultures and Inoculation

Preparation of root explant cultures was accomplished according to Haroon *et al.* (1993). Seeds of corn were treated for 3 min in 95% ethanol followed by a 10 min soak in 0.5% sodium hypochlorite and were transferred to sterile 1.5% water agar

Figure 1. A. *Ambrosia maritima,* B. *Calendula officinalis,* C. *Hyoscyamus muticus* and D. *Origanum majorana,* plants tested for nematicidal properties.

plates where they maintained for 3 d at 28C. A 5 mm of root tip was excised from each seedling and transferred to a sterile Petri plate (one root tip per plate) containing Gamborg's B-5 medium (Gibco, Grand Island, NY). All the plates were inoculated with one crushed cyst nematode of *Heterodera zeae* that had been collected from field crops located near the north coast of Egypt.

Preparation of Root Extracts

Five selected plants were grown in the greenhouse for 12 weeks. Roots were removed from pots and washed thoroughly in water and the weight determined for each plant. The roots were then surface sterilised in 0.5% NaOCl for 3 min and rinsed in three separate changes of sterilised water. The roots from each plant were homogenised in a food blender for 30 s in 100 ml sterilised water, the solution passed through a sterile 0.45 um micropore filter and then autoclaved for 20 min. Extracts not immediately used were stored at either 4C or frozen and used in all subsequent tests.

Eggs Hatch and Juvenile Survival Test

Eggs were removed from sterile cysts of *H. zeae* that had been propagated *in vitro* on corn root explants. The cyst was gently crushed in sterile water and 100 eggs were placed in sterile vials containing 1% extract in 5 ml sterile water for the 5 selected plants. Each treatment was replicated 5 times. Sterile water only was used as controls. Hatch and juvenile survival was recorded after 24 and 48 h.

Penetration and Development of Juveniles

Juveniles were exposed to 1% extracts of the five selected plants by adding it to Gamborg's B5 medium. Petri plates containing two roots of corn plant were prepared. After 40 d, the roots were removed from the agar, stained and examined for the presence of juveniles and different life stages of *H. zea*e.

Rate of Juveniles Penetration After Exposed to Different Concentration

Two different concentrations from the original extract were prepared, 0.5 and 1.0% in sterile water for each treatment. One hundred juveniles were exposed for 24 h. After exposure the nematodes were transferred to root explant culture plates, each with two corn roots. Each combination of extract source and concentration was replicated twice. After 48 h, the number of *H. zea*e in the roots were counted and the roots were weighed, stained in acid fuchsin and examined for nematode juveniles.

Data Analysis

Nematode counts were transformed $\log_{10}(x+1)$ before analysis. All data were subjected to an analysis of variance and means were separated using least significant difference test at $P \leq 0.05$.

RESULTS

Egg Hatch and Juveniles Survival Test

There was no hatch in any treatment except the control after 24 h. After 48 h, eggs had hatched in all treatments with very low numbers in extract of *A. maritima.* The greatest hatch occurred with *H. muticus* and *L. usitatissimum* while less eggs hatched in the *C. officinalis* and *O. vulgare* treatments. *A. maritima* was the only treatment that inhibit hatching strongly. Most of the freshly hatched juveniles were alive after 48 h in *H. maritima* and *L. usitatissimum* treatments, while most of the were dead in the *C. officinalis* and *O. vulgare* treatments. The few juveniles that hatched in *A. maritima* died less than 24 h after hatching (Table 1).

Table 1. Hatch and survival of juveniles of *Heterodera zeae* after 24 and 48 h exposure to root extracts of several medicinal plants.

Host	Hatching after 24 h	Juveniles after 48 h		
		Hatched eggs	Live	Dead
Hyoscyamus muticus	0	96 a	85 b	17 ab
Ambrosia maritima	0	5 d	0 c	5 c
Calendula officinalis	0	31 b	2 c	24 a
Linum usitatissimum	0	97 a	74 b	22 a
Origanum vulgare	0	12 c	2 c	10 ab
Sterile water	71	98 a	97 a	3 c

Means not followed by the same latter are different at $P<0.05$.

Penetration and Development of Juveniles

None of the five medicinal plants allowed complete development of the cyst nematode *H. zeae* inside the roots system.

A. martima did not support penetration or development of nematodes, only two juveniles were found in one replication. Low numbers of juveniles penetrated the root system of *O. vulgare* and *C. officinalis,* but these failed to develop beyond the second stage. In *H. muticus* and *L. usitatissimum* all the juveniles penetrated the root system and developed to third and fourth stage juveniles but none to mature adult females. No eggs were produced in any treatment except the control.

Rate of Juveniles Penetration After Exposed to Different Concentration.

Some of the juveniles exposed to 0.5% extract before inoculation onto the corn explants penetrated the roots in significantly lower numbers than the controls.

When 1.0% concentrations of extract were added to Gamborg's B5 medium, most of juveniles inoculated directly onto explants failed to penetrate the roots. A few juveniles exposed to the extracts of *A. maritima, H. muticus, L. usitatissimum*, *O. vulgare* or *C. officinalis* penetrated the roots, but in significantly lower numbers than the control. No juveniles that were exposed to 1.0% extracts penetrated the roots of *A. maritima* and *C. officinalis* (Table 2).

Table 2. *In vitro* hatch and penetration of *Heterodera zeae* on corn root explants after exposure for 48 h to different concentrations of several medicinal plants.

Host Plant	Concentration	
	0.5 ml/L	1.0 ml/L
Hyoscyamus muticus	2 c	1 c
Ambrosia maritima	2 c	0 c
Calendula officinalis	5 c	0 c
Origanum vulgare	8 bc	4 c
Linum usitatissimum	12 b	7 b
Zea mays	45 a	43 a

Means not followed by the same letter are different at $P<0.05$.

DISCUSSION

The effect of root extracts from some plants on hatching and the mortality of many plant parasitic nematodes species has been reported previously (Okada, 1971, Husain and Mahmood 1975, Haroon *et al.* 1993). In this study, five medicinal plants have shown some effect on hatching and development of *H. zea*e.

The most significant reduction in inhibition of egg hatch and mortality occurred in both concentrations of extracts of *A. maritima*, *C. officinalis* and *O. vulgare.* Also, after exposing the juveniles to these extracts for 48 h penetration and development was impaired. These plants appear to contain substances either directly toxic to the nematode or inhibitory to their normal invasion of and development in roots.

The study indicates that extracts from these plants have potential as biological nematicides and because these plants are already grown for their commercial value in some parts of the world, there is scope for their development for control and management of *H. zeae*.

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EFFECT OF SOLARISATION ON HETERODERA AVENAE AND WHEAT YIELD IN AL-QASSIM, SAUDI ARABIA*

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SUMMARY

The effect of soil solarisation on populations of *Heterodera avenae* and wheat growth was examined in heavily infested wheat field in Al-Qassim, Saudi Arabia. Solarisation was conducted by covering the soil with polyethylene sheets during the summer months (July-August). Uncovered plots were left to serve as a control treatment. Wheat seeds (cv. Yekora Rojo) were sown in both solarised and unsolarised plots in the following December. Solarisation treatments significantly ($P \leq 0.05$) reduced *H. avenae* populations and increased wheat yield. Plant growth and grain yield were 10 times greater than the control, while the number of white females on wheat roots decreased by 68%. This study provided evidence that soil solarisation could be implemented in programs designed for *H. avenae* management in Al-Qassim region, Saudi Arabia.

INTRODUCTION

The cereal cyst nematode, *Heterodera avenae,* is a serious problem in wheat production in Saudi Arabia (Al-Hazmi *et al.* 1994, 2001) and many countries world wide (Meagher 1977). Management practices to control this nematode in wheat include the use of crop rotation and nematicides in Al-Qassim, Saudi Arabia (Al-Rehiayani, 2002). However, increased social and legislative pressure to restrict the use of chemicals in controlling plant parasitic nematodes has created the awareness to evaluate alternative approaches of nematode management. Soil solarisation offers an alternative management method to control nematodes (Saha *et al*. 2007). Soil solarisation has been used effectively against nematodes and other soil borne pests (McSorley and McGovern 2000, Chellemi *et al*. 1997).

*Al-Rehiayani S, Belal M (2009) Effect of solarisation on *Heterodera avenae* and wheat yield in Al-Qassim, Saudi Arabia. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 233-236. (CIMMYT: Ankara, Turkey)

The objective of this study was to determine the effect of soil solarisation on *H. avenae* infecting wheat and wheat yield.

METHODS

The experiment was conducted during summer time (July to August) in wheat field in Al-Qassim, known to be heavily infested with *H. avenae*. The field was divided into plots, in a randomised complete block design of four replicates. Plots were 2 x 1 m and each had five crop rows. Clear polyethylene sheet (100 µm) was used to cover the soil and water was provided through drip irrigation system under the sheeting. Uncovered plots were left to serve as controls. Temperature were recorded in both covered (solarised) and uncovered (unsolarised) plots at depth of 15 cm. At the end of August, the polyethylene sheets were removed and the soil prepared for wheat cultivation. Wheat seeds cv. Yekora Rojo were sown in both solarised and unsolarised plots in the first week of December. All plots were sown and fertilised the same manner. One month before harvest root samples were carefully collected from each plot and number of white females and/or cysts on the roots were determined. Assessment of plant growth and yield components were made at physiological maturity. Data were subjected to analysis of variance and means were separated by Duncan's multiple range test.

RESULTS

Soil temperature was higher in solarised than the unsolarised plots by as much as 5 to 9C at depth of 15 cm (Table 1). Table 2 show the effect of the treatments on the *H. avenae* and wheat growth. The data indicate that soil solarisation can significantly ($P<0.05$) reduce the number of white females of *H. avenae* on the root system by 68%. Soil solarisation also improved plant growth, dry matter accumulation and grain yield. The difference in the plant height and shoot weight between the treatments was quite marked as can be seen in Figure 1.

Table 1. Soil temperature at depth of 15 cm recorded weekly in solarised and unsolarised plots during hot summer months (July and August).

Week	1	2	3	4	5	6	7	8
Solarised	50.6	4.8	43.6	44.2	53.9	52.5	52.8	51.6
Unsolarised	42.2	43.7	39.9	40.6	51.4	46.9	48.8	49.9

Table 2. Effect of soil solarisation on *Heterodera avenae* population and growth and yield components of wheat. Means in a column followed by the same letter are not different ($P<0.05$) according to Duncan's multiple range test.

Treatment	White females (no/g root)	Grain weight/m^2 (g)	Plant height (cm)	Shoot weight (g)
Solarised	12.5 b	328.8 a	37.7 a	11.6 a
Unsolarised	38.7 a	30.1 b	19.6 b	3.1 b

Figure 1. Effect of soil solarisation on wheat height and shoot weight. S, solarised and NS, not solarised.

DISCUSSION

This study provided evidence that soil solarisation using polyethylene sheets during hot summer months in Al-Qassim was effective in reducing populations of *H. avenae* in wheat. Soil solarisation increased soil temperature sufficiently to be responsible for reducing nematode population density in the soil (Sharma and Nene 1990). However, it has been found that the suppression of nematodes by solarisation is not as long lasting as can be achieved by fumigant nematicides (Saha *et al*. 2007). The efficacy of solarisation has been improved when was used in conjunction with organic amendment (McSorley and McGovern 2000). Therefore, additional research is needed to evaluate the efficacy of soil amendments available in the Al-Qassim region for enhancing the benefit of solarisation in managing *H. avenae*.

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IMPACT OF WINTER WHEAT, BARLEY, BROAD BEAN AND CLOVER AS PRECEDING CROPS ON POPULATION DENSITIES OF CORN CYST NEMATODE, *HETERODERA ZEAE,* ON CORN IN EGYPT*

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SUMMARY

Population dynamics of the corn cyst nematode, *Heterodera zeae* under stress of four cropping system regimes were studied under field conditions. Two non-hosts, (broad bean and Egyptian clover) and two hosts (barley and wheat) were used as winter crops preceding corn to form four regimes. It was found that nematode numbers fluctuated greatly according to the regime used. In broad bean-corn and Egyptian clover-corn rotations, the population densities of the nematode were low during the growing season of the winter crops (broad bean an Egyptian clover). Nematode densities gradually increased as corn was grown and peaked in September and October for juveniles and cysts, respectively. In barley-corn and wheat-corn rotations, the population densities of the nematode were relatively high during the winter season (February-May), leaving considerable initial populations for the next crop. Subsequently in the corn crop, the nematode multiplied rapidly reaching the peaks in August and September for juveniles and cysts, respectively. Accordingly, it is important to include non-hosts in the corn cropping sequence to minimise the nematode population and impact on corn.

INTRODUCTION

The corn cyst nematode, *Heterodera zeae* was discovered for the first time on corn (maize) in India by Koshy *et al*. (1971). Few years later it was recorded in many countries around the world. It was recorded in Egypt by Oteifa (1978, unpublished data), in Pakistan by Maqbool (1981) and in the USA by Sardanelli *et al*. (1981).

*Ismail AE (2009) Impact of winter wheat, barley, broad bean and clover as preceding crops on population densities of corn cyst nematode, *Heterodera zeae* on corn in Egypt. In 'Cereal cyst nematodes: status, research and outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 237-241. (CIMMYT: Ankara, Turkey)

Also, it was found infecting several poaceous weeds grown in association with corn plantation in India (Srivastava and Swarup 1975,Verma and Yadav 1978) and in Egypt (Ismail and Hasabo 1995). In Maryland, USA, Ringer *et al*. (1987) reported that *H. zeae* has other economic hosts including certain cultivars of barley, rice, sorghum, sugarcane and wheat as well as many cultivars of weeds. Moreover, in Pakistan, Maqbool (1981) reported that gram, citrus, pear and garlic as hosts to *H. zeae*. Nasira and Shahina (2007) found that mango, *Mangifera indica* is another host of *H. zeae*. Later in Egypt, certain cultivars of barley and wheat were reported as hosts (Abadir *et al*. 1989, Ismail and Youssef 1993, Ibrahim and Handoo 2007). Using suitable cropping systems for reducing nematode population and suppressing crop damage has been recommended for many years (Nusbaum and Ferris 1973). Selecting appropriate crops in a cropping sequence must be considered. Poor and non-hosts to a nematode species must be inserted in a cropping system design. Poaceous crops help in raising the population density of *H. zeae*; while the others decrease the nematode population (Ringer *et al*. 1987). Thus, using non-poaceous crops in a cropping sequence system during the winter season in infested soil may reduce the nematode population on the following summer crops even they are hosts. Therefore, the objective of this study was to evaluate the population dynamics of *H. zeae* under four cropping sequences including poaceous and non-poaceous crops.

METHODS

A field naturally infested with the corn cyst nematode, *H. zeae* was chosen at the Experimental Station Farm of the Faculty of Agriculture, Cairo University. The experimental field was divided into 24 plots, 14 x 12 m each. Each winter crop, including barley (*Hordeum vulgare*), broad bean (*Vicia faba*), Egyptian clover (*Trifolium alexandrinum*) and wheat (*Triticum aestivum*), was planted to six plots in a randomised block design. All the plots were maintained according to standard grower practices. Composite soil samples were collected from each plot for nematode assay at monthly intervals before and during the growing season (from January to May). After harvest, the experimental field plots were left fallow during June. All the plots were then re-planted with corn cv. Giza 2. During the corn growing season (July to October), soil samples were also taken from each plot at monthly intervals. The nematode juveniles were extracted from soil by sieving and Bearmann tray technique (Christie and Perry 1951). The cysts were extracted from the soil samples using a Fenwick can (Fenwick 1940).

RESULTS

Juvenile and cyst densities of *H. zeae* in four winter crops measured during the growing season are presented in Figure 1. In general, the nematode populations in barley and wheat were greater than the observed in broad bean or Egyptian clover. So, the juvenile populations in those plots planted with barley and wheat increased gradually to peak in April at 177 and 236 juveniles/250 g of soil, respectively. However, juvenile populations rapidly declined by the end of the growing season. The same trend was also observed in cyst numbers, peaking in May at 56 and 63 cysts/250 g of soil, respectively. By contrast, the juvenile and cysts densities in broad bean and Egyptian clover gradually declined during the growing season. No clear peaks in the juveniles or cysts numbers were observed (Figure 1).

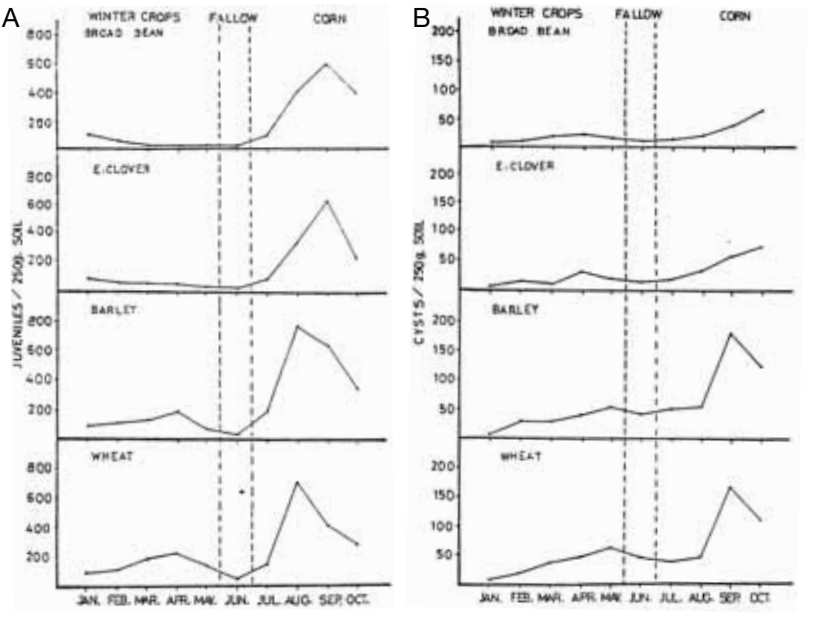

Figure 1. Population densities of *Heterodera zeae* juveniles (A) and cysts (B) under four cropping sequences.

A sharp drop in juveniles and cysts of the nematode occurred during the fallow period of June. However, barley and wheat had considerably higher cyst numbers than broad bean and Egyptian clover (Figure 1b).

With corn cultivation from July to October, the population density of *H. zeae* increased to a peak in August or September for juveniles and in September or October for cysts (Figure 1). However, nematode population densities were higher in corn after wheat or barley than after broad bean or Egyptian clover. The highest population densities of juveniles and cysts in corn planted after wheat and barley were 704 and 854 juveniles/250 g of soil, and 150 and 163 cysts/250 g of soil, respectively. Whereas, the maximum population densities of the nematode in corn afer broad bean and Egyptian clover were 573 and 630 juveniles/250 g soil and 68 and 90 cysts/250 g soil, respectively (Figures 1).

DISCUSSION

Population densities of *H. zeae* in the four winter crops studied indicated that broad bean and Egyptian clover are not hosts, while barley and wheat are suitable hosts. These results are consistent with the findings of Koshy *et al*. (1971), Srivastava and Swarup (1975), Bhargava and Yadav (1978), Aboul-Eid and Ghorab (1981), Bajaj *et al*. (1986), Ringer *et al*. (1987), Abadir *et al*. (1989), Ismail and Youssef (1993), Shahina and Erum (2007), and Ibrahim and Handoo (2007). In the non hosts, broad bean and Egyptian clover, the population densities declined during the winter growing season thus leaving low population densities in soil with less potential impact on a subsequent corn crop. Consequently, the nematode population remained

low in the subsequent corn and peaked later in the season. By contrast, in the hosts, barley and wheat, the nematode population increased during the winter growing season and left higher initial populations for the following corn crop. Therefore, the nematode increased more rapidly and to a greater density, peaking in a relatively early in the summer growing season. These findings mean that populations of *H. zeae* are strongly influenced by the cropping sequence. Thus, when broad bean-corn or Egyptian clover-corn rotations were practised, the population increase was insufficient to cause economic damage to corn; while when barley-corn or wheat-corn rotations were practised, the nematode increased strongly and could cause considerable damage. Similar results were reported by Srivastava and Sethi (1986), Ismail (1990) and Ismail and Youssef (1993). Accordingly, it is recommended that farmers grow non-host winter crops in rotation with corn to reduce *H. zeae* multiplication.

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Author Index

A

Abbad Andalouss F 45
Abdel-Mawgood AA 177
Ahmadi AR 79
Al Abed A 183
Al-Banna L 183, 208
Al-Dos AA 177
Al-Hazmi AS 56, 177
Al-Momany A 183
Al-Otayk S 177
Al-Rehiayani SM 177, 233
Alaoui Y 45
Andersson S 154

B

B'Chir MM 41
Bains NS 88
Belal M 233
Bert W 66
Bishnoi SP 143, 149
Bleve-Zacheo T 195
Blok V 189
Bolat N 11, 100, 149, 160
Braun HJ 11, 160

C

Çalışkan M 11
Chamberlain D 191
Chapman S 189
Chen SL 29, 149, 221
Chhibbar AK 200
Clark I 154
Çobanoğlu S 172
Cock PJ 189
Çolak Yılmaz Z 66
Colombo A 61

D

Dababat 172
Dawabah AAM 56, 177
De Sutter N 66
Deeren AM 66
Delibes A 195

E

Eastwood RF 166
Ebrahimi N 51
Elekçioğlu İH 11, 82, 100, 149, 160
Erginbaş-Orakcı G 100, 160

F

Fè Andrés M 195
Fosu-Nyarko J 191

G

Ghalandar M 51
Gitty M 51
Gowen SR 215
Gözel U 118

H

Hajihasani A 115
Hajji A 41
Hamroun O 130
Handoo Z 61
Haroon SA 227
Hekimhan H 100
Holgado R 154
Hou SY 29, 221

I

Imren M 82
Ismail AE 237

J

Jones JT 189
Jones MGK 191

K

Kaur DJ 88

Kanwar RS 149
Kazemi H 51
Kerry BR 215
Khoshkhabar Zh 51
Khurana P 200
Kılınç AT 11, 100, 160

L

Lafi HA 208
Lagudah E 166
Lewis JG 137
Li HL 29,149
Li HM 29
Li HX 29
Liu J 124
Lombardo S 61
López-Braña I 195

M

Ma P 29
Magnusson C 154
Matic M 137
McKay AC 23, 137
Melillo MT 195
Mitchinson S 215
Moens M 109
Mohammadi-Pour M 51
Mokjabli A 130
Mokrini F 45
Mor M 118
Motawei MI 177

N

Namouchi-Kachouri N 41
Nicol JM 3, 11, 29, 51, 82, 100, 149, 160, 172

O

Öcal A 82
Ogbonnaya F 149, 166
Oka Y 118
Othman E 227

P

Parihar A 73
Peng DL 29, 124, 149
Perera M 191

R

Rana J 191
Rao SB 204
Rapisarda C 61
Riley IT 23, 29, 221
Rivoal R 3
Rowe J 154

S

Sadder M 208
Sağlam HD 172
Şahin E 11, 100, 149, 160
Sashi V 200
Sharma A 204
Sharma AK 17
Sharma I 88
Sharma SK 73
Shoran J 17
Siddiqui AU 73
Simonetti E 195
Singh AK 17, 149
Singh Y 88
Smaha D 130
Smiley RW 35, 94
Sohu VS 88
Speigel Y 118
Subbotin SA 109

T

Tanha Maafi Z 51, 79, 115
Toktay H 11, 82
Troccoli A 45
Tülek A 100

U

Umarao 200, 204

V

Vandenbossche B 66
Veronico P 195
Viaene N 66, 109, 172

W

Waeyenberge L	109
Wesemael W	172
Wu HY	124

Y

Yan GP	94
Yıldırım AF	11, 100, 160
Yorgancılar A	11, 100, 160
Yorgancılar Ö	11, 100
Youssef RM	227
Yu GL	124

Z

Zhang GM	124
Zhang JJ	191

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