Nematology, 2001, Vol. 3(4), 379-382

Short communication

Pathotype characterisation of the cereal cyst nematode, *Heterodera avenae*, in Saudi Arabia

Ahmad S. AL-HAZMI [1,*], Roger COOK [2] and Ahmed A.M. IBRAHIM [1]

The cereal cyst nematode (CCN), *Heterodera avenae* Woll., has been reported on wheat and other cereals in many countries with different climatic types throughout the world (Holdeman & Watson, 1977; Meagher, 1977; Sturhan & Rumpenhorst, 1996). In 1987, the nematode was also reported from irrigated wheat fields in Saudi Arabia (Youssif, 1987). Since then, *H. avenae* has been increasingly detected in Saudi Arabia year after year and has become recognised as a damaging pathogen of wheat and barley, especially in Al-Kharj, Hail and Al-Gassim, the three major wheat-producing regions (Al-Hazmi, 1992; Al-Hazmi *et al.*, 1994). Growth and physiology of infected wheat and barley are adversely affected (Al-Yahya *et al.*, 1998), and yield loss is as much as 92% in heavily-infested sites of some wheat fields (Ibrahim *et al.*, 1999).

Some records of cereal cyst nematode may refer to other species other than *H. avenae*. For example, comparative biochemical and morphological studies made by Sturhan and Rumpenhorst (1996) on numerous *H. avenae* and *H. avenae*-resembling populations from several geographic origins in eastern Europe and west Asia proved that these populations are, in fact, *H. filipjevi* Madzhidov. However, the protein pattern of the Saudi population from Al-Kharj region closely agreed with that of 'typical' *H. avenae* (Sturhan & Rumpenhorst, 1996). Cotten (1967) reported that more than three pathotypes of CCN were present in England and Wales and, since then, variations in virulence of CCN populations on different cereal genotypes have been described from Asia (Mathur *et al.*, 1974; Shimizu *et al.*, 1987; Dhawan, 1988; Zheng-Jing *et al.*, 1997) and many other countries (Swarup & Sosa-Moss, 1990; Rivoal & Cook, 1993). Characterisation of the cereal cyst nematode species and pathotypes is essential for resistance breeding and nematode management programmes.

The objective of this study was to characterise the pathotype of three populations of *H. avenae* collected from the three major wheat-producing regions of Saudi Arabia.

Materials and methods

Tests were made in environment-controlled growth chambers in Wales, UK (two tests) and Riyadh, Saudi Arabia (three tests) to determine the pathotype of *H. avenae* populations from three major wheat-producing regions in Saudi Arabia (Al-Kharj, Hail and Al-Gassim). In all tests, the International Test Assortment of cereals (barley, oat and wheat) (Table 1) was used, with two locally-grown cultivars of barley (Beecher and CC 189) and wheat (Yecora rojo and West bred) as known susceptible controls. *H. avenae*-infested soils were collected at the end of the growing season from wheat fields in the three regions. Cysts were extracted from soil by flotation (Shepherd, 1986) and identified as *H. avenae* on the basis of morphometric and morphological features according to Mulvey and Golden (1983) and Golden (1986). The cysts were then stored in steam-sterilised white sand in a cold room (2°C) until use. Whenever needed, cysts were re-extracted and crushed, and their contents suspended in water and aerated before transfer to nylon sieves in Petri plates or hatching cups. These were kept in an incubator at 5/15°C (18 h dark, 6 h light) for hatching and second-stage juveniles (J2) collected daily, stored in water at 2°C and then aerated for use as inoculum.

[1] *Plant Protection Department, College of Agriculture, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia*
[2] *Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Wales SY23 3EB, UK*
[*] Corresponding author, e-mail: aasamie@ksu.edu.sa
Received: 31 January 2001
Accepted for publication: 4 March 2001
Keywords: barley, oat, resistance, virulence, wheat.

Table 1. *Mean numbers (± standard deviation) of white females per plant produced by three Saudi populations of* Heterodera avenae *on cereal differential cultivars, 60 days after inoculation.*

Cereal cultivars	Origin of nematode population			Reaction
	Al-Kharj	Hail	Al-Gassim	
Barley				
Varde	18 ± 4[1)]	36 ± 16	46 ± 17	S
Emir	25 ± 5	22 ± 6	42 ± 7	S
Ortolan	0	2 ± 1	0	R
KVL 191	<1	<1	<1	R
Siri	0	0	0	R
Morocco	0	0	0	R
Bajo Aragon 1-1	0	0	0	R
Herta	19 ± 14	27 ± 7	39 ± 7	S
Martin 403-2	0	0	0	R
La Estanzuela	33 ± 13	42 ± 17	61 ± 6	S
Oats				
Nidar II	<1	<1	0	R
Sun II	0	0	0	R
Silva	0	0	0	R
CI 3444 (Pusa Hybrid)	0	0[2)]	0	R
Avena sterilis (I 376)	0	0	0	R
Wheat				
Capa	41 ± 6	46 ± 27	30 ± 4	S
Loros	9 ± 1	11 ± 6	15 ± 4	S
Iskamish K-2-light	6 ± 0	6 ± 3	10 ± 1	S
AUS 10894	11 ± 4	10 ± 4	10 ± 6	S
Psathias	22 ± 7	14 ± 8	19 ± 1	S
Locally-grown cultivars (controls)				
Barley				
Beecher	14 ± 9[3)]	17 ± 16	64 ± 4	S
CC 189	23 ± 9	10 ± 8	53 ± 9	S
Wheat				
Yecora rojo	26 ± 8	32 ± 13	46 ± 6	S
West bred	56 ± 9	41 ± 17	49 ± 3	S

Values for Al-Kharj and Hail are means of six replicates except [1)]four replicates and [2)]five replicates from two separate tests in Riyadh and Wales and for Al-Gassim of three replicates from one test only in Riyadh.

In each test, clean plastic pots (100 cm^3) were filled with autoclaved soil mixture (2 loam : 1 sand), and 7-day old cereal seedlings were transplanted individually into the pots, with three replications of each cereal cultivar. Plants were grown in a controlled-environment chamber for 7 days at 12/15°C (14 h dark/10 h light), and then for 3 days at 7/12°C (8 h dark/16 h light) before inoculation. Aliquots of aerated J2 suspension were used to inoculate each seedling with 175 J2 on three occasions over 4 days, a total of 525 J2 per plant. After inoculation, plants were grown for 7 days at 10/12, 7 at 12.5/17.5, 14 at 15/20 and 32 at 17.5/22.5°C, with 8 h dark/16 h light. Soil in the pots was kept at 60% water capacity until the end of experiment.

Sixty days after inoculation, plants were removed from pots and roots washed in a gentle stream of tap water. White females attached to the roots were counted under a stereoscopic microscope, and the mean number of white females/plant calculated. Population/plant combinations with up to three white females were defined as avirulent/resistant, and those with more than three as virulent/susceptible (Mathur *et al.*, 1974; Ireholm, 1994). Responses of the tested differentials were compared with those given by Andersen and Andersen (1982) and Rivoal and Cook (1993) to characterise the pathotype.

Results

The locally-grown cultivars of barley and wheat (controls) were fully susceptible to all the tested *H. avenae* populations. The responses of each differential cultivar were similar in all tests with all populations (Table 1). All tested populations lacked virulence on the oat differentials. Wheat differential Capa was as susceptible as the local controls and Psathias was also susceptible. Loros, Iskamish K-2-light and AUS 10894 were less susceptible than the controls. The barley differentials were either as susceptible as the local controls (Varde, Emir, Herta and La Estanzuela) or resistant (Ortolan, KVL 191, Siri, Morocco, Bajo Aragon 1-1 and Martin 403-2).

Discussion

The susceptibility of the controls confirmed the viability and virulence of the inoculum. The similar responses of the differentials indicate that the three tested Saudi populations of *H. avenae* are predominately the same pathotype. According to the scheme of Andersen and Andersen (1982) and its subsequent revisions (Rivoal & Cook, 1993), three primary groups of pathotypes are distinguished by the reactions of barley differentials. Within each group, additional pathotypes are identified by the reactions of additional barleys, oats and wheats. Based upon this scheme, the Saudi populations are in the Ha1 group: the reactions of the barleys classify them as either Ha11 or Ha21. Oat Sun II is susceptible to Ha11 but resistant

to Ha21, and the reactions of the other oats are also consistent with the Saudi populations being Ha21. However, the degree of susceptibility of all the wheat differentials distinguishes the Saudi populations from other pathotypes in the Ha1 group. The partial susceptibility of three of the wheats and the new combination of virulence in these populations supports the concept that populations of *H. avenae* may be highly heterozygous and pure pathotypes might be unusual (Cook & Rivoal, 1998).

Although oats have been considered as the preferred hosts of *H. avenae*, similar observations regarding lack of virulence on oats have been reported in a number of *H. avenae* populations from several countries in Asia and Europe (Mathur *et al.*, 1974; Ireholm, 1994; Peng & Cook, 1996). Ireholm (1994) suggested that a pathotype-specific reaction on oats could be valuable for improving the classification and that it would be desirable to test more spring and winter oats as well as other cultivated species of *Avena*.

The origins of the *H. avenae* populations in these centres of wheat production are not known. Although wheat and barley have been traditionally grown for a long time in Saudi Arabia, the great increase of cultivated area and production has only occurred more recently as a consequence of governmental policy and availability of irrigation and modern technology. Oat crops have not been grown in the country, particularly in these regions. However, wild species of oats might occur as weeds in crops and local flora. The *H. avenae* populations from the three regions appear to have the same novel virulence phenotype and may be heterogenous for wheat virulences. It may be that these populations are indigenous or introduced from a single source. Results of this work indicate the need for additional screening of wheat as a source of germplasm for resistance breeding programmes.

Acknowledgements

The authors are grateful to the British Council in Riyadh, Saudi Arabia for supporting the studies conducted in Wales, UK, and to the Nordic Gene Bank, Sweden for kindly supplying seeds of the International Test Assortment of cereals.

References

AL-HAZMI, A.S. (1992). Status of plant nematology in Saudi Arabia. In: Maqbool, M.A. & Zaki, M.J. (Eds). *Expert consultation on plant nematode problems and their control in the near east region. Second International Meeting on Plant Nematology, 22-26 November, 1992, Karachi, Pakistan*, p. 70. [Abstr.]

AL-HAZMI, A.S., IBRAHIM, A.A.M. & ABDUL-RAZIG, A.T. (1994). Occurrence, morphology and reproduction of *Heterodera avenae* on wheat and barley in Saudi Arabia. *Pakistan Journal of Nematology* 12, 117-129.

AL-YAHYA, F.A., ALDERFASI, A.A., AL-HAZMI, A.S., IBRAHIM, A.A.M. & ABDUL-RAZIG. A.T. (1998). Effects of cereal cyst nematode on growth and physiological aspects of wheat under field conditions. *Pakistan Journal of Nematology* 16, 55-62.

ANDERSEN, S. & ANDERSEN, K. (1982). Suggestions for determination and terminology of pathotypes and genes for resistance in cyst-forming nematodes, especially *Heterodera avenae*. *EPPO Bulletin* 12, 379-386.

COOK, R. & RIVOAL, R. (1998). Genetics of resistance and parasitism. In: Sharma, S.B. (Ed.). *The cyst nematodes*. London, UK, Chapman & Hall, pp. 322-352.

COTTEN, J. (1967). Cereal root eelworm pathotypes in England and Wales. *Plant Pathology* 16, 54-59.

DHAWAN, S.C. (1988). 'Katyil', a resistant wheat cultivar, susceptible to Indian population of cereal cyst nematode, *Heterodera avenae*. *Indian Journal of Nematology* 18, 140.

GOLDEN, A.M. (1986). Morphology and identification of cyst nematodes. In: Lamberti, F. & Taylor, C.E. (Eds). *Cyst nematodes*. New York, NY, USA, Plenum Press, pp. 23-45.

HOLDEMAN, Q.L. & WATSON, T.R. (1977). *The oat cyst nematode,* Heterodera avenae, *a root parasite of cereal crops and other grasses*. California, CA, USA, Department of Food and Agriculture Bulletin, 82 pp.

IBRAHIM, A.A.M., AL-HAZMI, A.S., AL-YAHYA, F.A. & ALDERFASI, A.A. (1999). Damage potential and reproduction of *Heterodera avenae* on wheat and barley under Saudi field conditions. *Nematology* 1, 625-630.

IREHOLM, A. (1994). Characterization of pathotypes of cereal cyst nematodes, *Heterodera* spp., in Sweden. *Nematologica* 40, 399-411.

MATHUR, B.N., ARYA, H.C., MATHUR, R.L. &. HANDA, D.K. (1974). The occurrence of biotypes of the cereal cyst nematode (*Heterodera avenae*) in the light soils of Rajasthan and Harayana, India. *Nematologica* 20, 19-26.

MEAGHER, J.W. (1977). World dissemination of the cereal-cyst nematode (*Heterodera avenae*) and its potential as a pathogen of wheat. *Journal of Nematology* 9, 9-15.

MULVEY, R.H. & GOLDEN, A.M. (1983). An illustrated key to the cyst-forming genera and species of Heteroderidae in the Western Hemisphere with species morphometrics and distribution. *Journal of Nematology* 15, 1-59.

PENG, D.L. & COOK, R. (1996). Observations on pathotypes of cereal cyst nematode (*Heterodera avenae*) in China. *Afro-Asian Journal of Nematology* 6, 176-178.

RIVOAL, R. & COOK, R. (1993). Nematode pests of cereals. In: Evans, K., Trudgill, D.L. & Webster, J.W. (Eds). *Plant*

parasitic nematodes in temperate agriculture. Wallingford, Oxon, UK, CAB International, pp. 259-304.

SHEPHERD, A.M. (1986). Extraction and estimation of cyst nematodes. In: Southey, J.F. (Ed.). *Laboratory methods for work with plant and soil nematodes*. London, UK, Ministry of Agriculture, Fisheries & Food, Her Majesty's Stationery Office, pp. 31-49.

SHIMIZU, K., MOMOTA, Y., OHSIMA, Y. & MITSUI, Y. (1987). Races of the cereal cyst nematode (*Heterodera avenae*) in Japan. *Japanese Journal of Nematology* 17, 42-47.

STURHAN, D. & RUMPENHORST, H.J. (1996). Untersuchungen über den *Heterodera avenae*-Komplex. *Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft Berlin Dahlem* 317, 75-91.

SWARUP, G. & SOSA-MOSS, C. (1990). Nematode parasites of cereals. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*. Wallingford, Oxon, UK, CAB International, pp. 109-136.

YOUSSIF, G.M. (1988). Effect of *Heterodera avenae* populations in infested field patches of wheat cultivation on plant growth and yield. *Abstracts of the Tenth Symposium on the Biological Aspects of Saudi Arabia, 20-24 April 1987, Jeddah, Saudi Arabia*, pp. 183. [Abstr.]

ZHENG-JING, W.U., CHENG-HURUI, FANG-ZHONG, D.A., ZHENG, J.W., CHENG, H. & FANG, Z.D. (1997). Pathotype of cereal cyst nematodes (*Heterodera avenae*) on wheat in Shanxi and Anhui, China. *Acta Phytopathologica Sinica* 27, 309-314.