The importance, biology and management of cereal cyst nematodes
(Heterodera spp.)

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Abstract
Cereals are exposed to biotic and abiotic stresses. Among the biotic stresses, plant-parasitic nematodes play an important role in decreasing crop yield. Cereal cyst nematodes (CCNs) are known to be a major constraint to wheat production in several parts of the world. Significant economic losses due to CCNs have been reported. Recognition and identification of CCNs are the first steps in nematode management. This paper reviews the current distribution of CCNs in different parts of the world and the recent advances in nematode identification. The different approaches for managing CCNs are also discussed.

Keywords: Cereals, cyst nematodes, identification, management, control

INTRODUCTION
Among the cereals, wheat (Triticum aestivum and T. durum) and barley (Hordeum vulgare) occupy the most prominent position in terms of production, acreage and source of nutrition, particularly in developing countries (Nicol et al., 2011). By 2030, the world production of cereals is expected to increase to 8 billion tonnes; that of T. aestivum is estimated to increase from 584 million tonnes (average 1995-1999) to 860 million tonnes (Hossain et Teixeira Da Silva, 2012). In Morocco, cereals occupy 75% of the cultivated area and account for 10–20% of the agricultural gross domestic product (Benabdelouahab et al., 2016). Cereal production of the season 2013-2014 was estimated at 6.8 million tonnes and includes 4.42 million tonnes of common wheat, 0.5 million tonnes of durum wheat and 0.4 million tonnes of barley, ranking 15th among the cereal producing countries (Anonymous, 2014). Productivity of soft wheat (T. aestivum), durum wheat and barley is low, due to biotic and abiotic stresses. Consequently, Morocco is not self sufficient in wheat production most of the years and imports bread wheat for its domestic consumption (Balagh et al., 2013). Cereal production occurs in most parts of the country but is mainly concentrated in 6 regions, each contributing differently to a certain type of cereal (Table 1).

<table>
<thead>
<tr>
<th>Region</th>
<th>Soft wheat</th>
<th>Durum wheat</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tadla</td>
<td>11.4</td>
<td>5.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Gharb</td>
<td>25.6</td>
<td>4.4</td>
<td>-</td>
</tr>
<tr>
<td>Chaouia</td>
<td>10.7</td>
<td>22.4</td>
<td>15.6</td>
</tr>
<tr>
<td>Saïss</td>
<td>14.6</td>
<td>16.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Haouz</td>
<td>6.1</td>
<td>12.2</td>
<td>22.4</td>
</tr>
<tr>
<td>Oriental</td>
<td>-</td>
<td>-</td>
<td>11.0</td>
</tr>
<tr>
<td>Total</td>
<td>86.4</td>
<td>60.5</td>
<td>57.9</td>
</tr>
</tbody>
</table>

THE CEREAL CYST NEMATODES (CCNS)
Heterodera is a very important genus of the family Heteroderidae (Nematoda). Members of this genus are obligate parasites and different species attack different crops, often causing great economic damage. The genus is particular among nematode genera because of the ability of the female to transform into a tough brown cyst, which protects the eggs formed within her body.

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DISTRIBUTION AND IMPORTANCE

Cereal cyst nematodes form a group of several closely related species that have been documented as causing economic yield losses in wheat production systems in several parts of the world, including West Asia, North Africa, Europe, Australia and the United States of America (Rivoal et Cook, 1993; Nicol et Rivoal, 2008; Sahin et al., 2009; Yan et Smiley, 2009). Twelve species affect roots of cereals and grasses (Nicol et Rivoal, 2008; Subbotin et al., 2009; Yan et Smiley, 2009), among which three species, viz. H. avenae, H. latipons and H. filipjevi, are considered the most economically important, and sometimes coexist (Rivoal et Cook, 1993; Abidou et al., 2005; Mc Donald et Nicol, 2005).

Out of these three species, H. avenae is the most widely distributed and damaging species in temperate wheat producing regions throughout the world (Rivoal et Cook, 1993). It is known as a major production constraint of cereals in Europe (Rivoal et Cook, 1993), Australia (Brown, 1984), India (Khan et al., 1990; Singh et al., 2009), North America (Miller, 1986), and in several countries of North Africa and West Asia (Sikora, 1988; Al-Yahya et al., 1998; Nicol et al., 2011). Heterodera latipons has been found in the Mediterranean region (Greco, 1994), but has also been detected in the temperate continental climates of Southern Russia, Ukraine, Central Asian Republics (Subbotin et al., 1996), Iran (Talatschian et al., 1976), Europe (Sabova et al., 1988), and Canada (Sewell, 1973). Heterodera filipjevi has been found in more continental climates such as Russia, Tadzhikistan (Subbotin et al., 1999), but also in Pakistan, Turkey (Rumpenhorst et al., 1996), Norway (Holgado et al., 2004), and USA (Smiley et al., 2008). Several other species of Heterodera (e.g., H. hordecalis, H. zeae and H. bifenesstra) are reported on wheat but are not considered to be of major economic importance (Smiley et Nicol, 2009; Lambardo et al., 2009; Sharma et al., 2009).

In Morocco, H. avenae was reported for the first time in an irrigated wheat field in 1951 (Ritter, 1982). It has been increasingly detected over the last few years and is recognized as a damaging pathogen of wheat and barley in most cereal growing areas, especially in Zaer, Sais, Chaouia and Doukkala (Meskine et al., 2003; Znasni, 2003; Mokrini et al., 2009, 2012a). Recently, H. latipons was found for the first time in the wheat growing area of Sais region (Ain Jmaa) (Mokrini et al., 2012b). However, H. filipjevi has never been reported in Moroccan cereal fields.

Cereal cyst nematodes can cause considerable yield reduction, especially in temperate climates and semi-arid regions where they can aggravate drought stress (Rivoal et Cook, 1993). Yield losses caused by CCNs can be up to 90% in severely infested fields (Rivoal et Cook, 1993; Riley et al., 2009). Several authors have reported that water stress is one of the key environmental conditions that can exacerbate damage caused by CCNs (e.g., Nicol et al., 2011). Yield losses due to H. avenae on wheat are reported to be 40-92% in Saudi Arabia (Ibrahimi et al., 1999), 10% in China (Peng et al., 2009), 40-50% in Morocco (Ramah, 1994), and 23-50% in Australia (Meagher, 1972). It has been calculated that H. avenae is responsible for annual yield losses of 72 million Australian dollars in Australia (Brown, 1981). Several studies have shown the economic importance of H. latipons and H. filipjevi (Nicol et al., 2006; Hajihasani et al., 2010). Nicol et al. (2006) showed that H. filipjevi infestation can be highly destructive to Triticum spp. Hajihasani et al. (2010) performed a study on the effects of H. filipjevi on the yield and growth parameters of T. aestivum cv. Sardari (bread wheat). The authors concluded that a population density of 20 eggs or second-stage juveniles (J2) per g of soil reduced grain yield up to 55%, root dry weight up to 70%, aerial shoot dry weight up to 48%, spike height up to 36%, and plant height up to 32%. In addition to yield loss of wheat, Philis (1988) reported up to 50% yield loss of barley in fields infested with H. latipons in Cyprus. In Turkey, H. filipjevi was found in all wheat growing areas in the East Anatolia Region, the Central Anatolian Plateau (CAP) and transition zone (Sahin et al., 2009, Toktay et al., 2015) with an estimate of yield loss up to 50% in several rain-fed winter locations (Nicol et al., 2006).

IDENTIFICATION OF HETERODERA SPECIES

In the H. avenae group, as in each Heterodera group, only minor morphological and morphometrical differences distinguish species from each other. The structures of the cone top of the cyst, including fenestra, bullae and underbridge are used to separate the species. However, the increasing number of species in this group makes reliable morphological identification more difficult and time consuming (Subbotin et al., 2003). Molecular identification can confirm traditional identification, especially for morphologically closely related species. Several molecular techniques can be used for the separation of species and populations of the H. avenae group, including species-specific primer sets for differentiating H. avenae, H. filipjevi, and H. latipons (Toumi et al., 2013a, Toumi et al., 2013b; Yan et al., 2013; Waeyenberge et Viaene, 2015), sequences of ITS-rDNA (Ferris et al., 1994; Bekal et al., 1997; Subbotin et al., 2003) and restriction fragment length polymorphism (RFLP) (Subbotin et al., 1999).

CEREAL CYST NEMATODE PATHOTYPES

The term “pathotype” refers to a biological entity (nematode population) that is distinguished by its inherent capacity (or inability) to multiply on a given host genotype with one or more genes for resistance (Trudgill, 1986). Numerous schemes have been developed to classify nematodes according to their parasitic capabilities (Triantaphyllou, 1987). The pathotype scheme for CCNs is based on their multiplication on host differentials of barley, oats and wheat cultivars in the International Cereal Test Assortment developed by Andersen and Andersen (1982). The test uses 12 barley (Hordeum vulgare), six oat (Avena sativa), and six wheat differential cultivars to define pathotypes of H. avenae. This scheme distinguishes three primary groups, based on host resistance reactions of barley cultivars carrying the resistance genes Rha1, Rha2, and Rha3. In Europe, North Africa, and Asia, most populations of H. avenae belong to groups 1 (Ha1) and 2 (Ha2) (Al-Hazmi et al., 2001; Cook et Noel, 2002; Znasni, 2003). Pathotypes of group 3 are mostly found in Australia, Europe, and North Africa (Rivoal et Cook, 1993). In
Morocco, Znasni (2003) reported the presence of two pathotype groups (Ha1 and Ha2). The characterization of the CCNs species and pathotype is essential for developing resistance in breeding and applying appropriate cultivars in nematode management programs.

BIOLOGY

The life cycle of members of the *H. avenae* group involves various stages, including the egg, four juvenile stages, and the adult nematode (Subbotin et al., 2010). The species completes one generation per growing season (Rivoal et Cook, 1993); to complete its life cycle, the nematode requires between three and four months under low soil temperature (5-15°C) and high soil moisture (Smiley et Nicol, 2009). Cyst nematodes are characterized by the developing female swelling and becoming a cyst, which contains several hundred eggs. Within the cyst, eggs may remain dormant in soil for several years. Each egg contains a single first-stage juvenile (J1), which moults inside the egg to become a second-stage juvenile (J2) (Figure 1). Emergence of J2 from eggs enclosed in brown cysts requires a period of dormancy (diapause) that differs among species and climatic region (Smiley et Nicol, 2009). The induction or suppression of dormancy by different temperatures regulates the hatching of juveniles. For *H. avenae*, two ecotypes appeared to differ in the induction or suppression of dormancy (diapause) by different thermal conditions (Rivoal, 1986). In Mediterranean climates, the diapause is acting when the climate is hot and dry; diapause is suppressed when the soil temperature falls and moisture rises (Rivoal et Cook, 1993).

Further research with North African populations (Algeria, Morocco and Tunisia) demonstrated hatching schemes relevant to the Mediterranean ecotype, with a higher optimum of hatching temperatures, which could express adaptation of populations to warmer climatic conditions (Rivoal et Nicol, 2009). Scholz and Sikora (2004) demonstrated that the hatch of *H. latipons* in Syria was similar to the Mediterranean ecotype of *H. avenae* from France and southwest Spain. By contrast, *H. filipjevi* originating from Turkey does not show any diapause as the juveniles hatch immediately at the beginning of the winter wheat growing period (Sahin et al., 2009).

Second-stage juveniles of CCNs invade just behind the growing apex of the root tip (Von Mende et al., 1998) and then pass through cells towards the stele where they initiate the development of a cluster of multinucleate feeding cells called a syncytium (Baldwin et Mundo-Ocampo, 1991). The J2 then go through a moult to the third-stage (J3). The syncytium provides food for the development and maturation of the juveniles to adult stage. The strong sexual dimorphism develops after the fourth-stage (J4). The adult males become vermiform and leave the root (Sijmons et al., 1994), whereas the females swell into a white lemon-shaped body that protrudes out of the surface of the root. After mating, the females produce eggs that are kept within their bodies. When all eggs have formed, the female dies and becomes a cyst, detached from the root (Smiley et Nicol 2009).

SYMPTOMS

Aboveground symptoms caused by members of the *H. avenae* group include yellowing, poor tillering, stunting and patchy growth of the host plants. There may be a burning of the leaves, similar to that caused by drought conditions. This is due to the nematode interfering with the
metabolic balance of the plant and inhibiting hydrostatic water pressure, which results in wilting (Griffin, 1988). The symptoms caused by members of the *H. avenae* group on the roots are different depending on the host. Wheat attacked by *H. avenae* shows increased root production such that the roots have a ‘bushy knotted’ appearance, usually with several females visible at each knot (Rivoal et Cook, 1993). While *H. avenae* is far more common than *H. filipjevi*, these two species have similar host ranges and cause similar symptoms and economic losses (Smiley, 2009). Root symptoms often do not become recognizable until one to three months after planting, depending on climatic conditions and spring or winter wheat growth habits (Smiley et Nicol, 2009). Root systems of wheat and barley plants infested with *H. avenae* include elongation of the main root, bunched tips of rootlets and a knotted appearance due to cysts. Infected oat roots appear ‘ropy’ and swollen (Smiley et Yan, 2010). Root symptoms of *H. latipons* are different from those seen with *H. avenae*, with no characteristic “knotting” caused by excessive production of lateral roots at the site of infection (Mor et al., 1992).

**MAJOR METHODS OF CONTROL**

Reducing yield loss caused by cereal cyst nematodes requires control of CCNs below the damage threshold or growing non-susceptible crops. This requires observations of population dynamics and yield losses on representative local cultivars under natural field conditions (Smiley et Nicol, 2009). Cultural practices based on rotations of non-hosts (non-cereals) and clean fallow can effectively control CCNs (Dababat et al., 2015). Singh et al. (2009) showed that *H. avenae* population densities decreased by 70% after rotation with non-host crops like carrot (*Daucus carota*), fenugreek (*Trigonella foenum-graecum*), and onion (*Allium cepa*), or by fallow and summer ploughing. Nematicides can be applied, but are not preferred by the farmers because of the high cost per unit area in wheat (Dababat et al., 2015). However, when the nematode population in the soil is high, and other management approaches are inadequate, chemical control can bring the *H. avenae* population below damage threshold levels (Hague et Gowen, 1987).

Chemical control of nematodes is often considered economically and environmentally unacceptable (Viaene et al., 2013), so development of microbial antagonists for CCNs might be one of the few remaining alternatives (Riley et al., 2010). A range of microorganisms has been investigated as potential biocontrol agents for CCNs including, *Pochonia chlamydosporium*, *Trichoderma longibrachiatum* and *Purpureocillium lilacinum* (Kerry et al., 1984; Zhang et al., 2014). Likewise, some bacteria have been shown to offer potential as biocontrol agents. A bacterium similar to *Pasteuria spp.* was able to parasitise *H. avenae* and was shown to prevent 38 to 56% of the juveniles from invading roots (Davies et al., 1990). Bansal et al. (1999) showed that *Azotobacter chroococcum* reduced cyst formation by 48%. However, little information has been gathered on biological control of CCNs in recent years.

Soil solarization offers an alternative management method to control nematodes (Viaene et al., 2013). Al-Rehiyani and Belal (2009) showed that soil solarization using polyethylene sheets during hot summer months in Al-Qassim (Saudi Arabia) was effective in reducing populations of *H. avenae* in wheat.

The potential use of biofugation derived from plants or organisms is an alternative non-chemical for controlling many plant-parasitic nematodes. For CCNs, there is no information about their control by this approach. Haroon et al. (2009) used some medicinal plants (root extracts) for controlling *Heterodera zeae* and they concluded that *Calendula officinalis*, *Ambrosia maritima* and *Oriiganum vulgare* significantly reduced the hatching of eggs and mortality of *H. zeae* compared to the control. In addition, several studies have demonstrated the potential of brassicaceous crops to control the potato cyst nematode *Globodera pallida* in potato production (Ngala et al., 2015).

One of the most economic, environmental and promising methods of managing CCNs is the use of resistant wheat germplasm (Dababat et al., 2015). Many sources of resistance in wheat germplasm have been reported (Smiley et Nicol, 2009). Resistance sources around the world were obtained from wild wheat relatives through breeding programme (Ogbonnaya et al., 2001). At least nine single dominant resistance genes (*Cre* genes) have been found, many of which derive from wild relatives of wheat (Dababat et al., 2015). Six *Cre* genes (*Cre2 to Cr7*) were derived from *Aegilops* spp. (Jahier et al., 2001); other resistance genes were derived from *T. aestivum* (*Cr1* and *Cr8*) and *rye* (*Secale cereale*) lines (*CreR*) (Barloy et al., 1996). Sources of resistance to *H. avenae* have been collated and reviewed and, where possible, have had their genetic location and gene designation reported (Table 2) (Rivoal et al., 2001; Nicol et al., 2003; Mc Donald et Nicol, 2005; Nicol et Rivoal, 2008; Vanstone et al., 2008).

Some sources of resistance currently used to control *H. avenae* in wheat and barley in Australia have been found to be effective against *H. latipons*. The Iraqi landrace AUS4930 is resistant to both *H. australis* (= Australian pathotype Ha1) and the Turkish *H. filipjevi* (pathotype HF1) (Nicol et Rivoal, 2000). However, the use of resistance requires a sound knowledge of the virulence spectrum of the targeted species. Several studies showed that the wheat cultivars resistant to populations of *H. avenae* in one region were fully susceptible to populations of the same species in other regions (Bonfil et al., 2004; Smiley et Nicol, 2009).

**CONCLUSION**

Accurate identification of the nematode species present in the field and knowledge of their population density are essential when designing effective control measures. As is common for other nematode species, CCNs are traditionally identified on the basis of their morphology and morphometrics. Unfortunately, this is time-consuming and hardly applicable when species-mixtures need to be identified and quantified. However, it was shown that DNA-based methods can be excellent tools complementing the traditional identification. Genetic resistance, biological agents, cultural practices, and chemical strategies can all be part of a management strategy, but each of them has its
limitations. However, the use of resistant cereal lines or cultivars is considered the most economically feasible and environmentally sustainable method. Advances in research on CCNs in cereals are exchanged and discussed regularly during the meetings of the International Cereal Nematode Initiative (ICNI). The 6th ICNI meeting will take place in September 2017, Agadir, Morocco.

REFERENCES


Table 2: Main sources of genes used in bread wheat (*Triticum aestivum*) for resistance to the cereal cyst nematode *Heterodera avenae* (after Smiley et Nicol, 2009)

<table>
<thead>
<tr>
<th>Cereal species</th>
<th>Genotype</th>
<th>Resistance gene and location</th>
<th>Use in cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. aestivum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. aestivum</td>
<td>Loros, AUS10894</td>
<td><em>Cre1</em> (formerly <em>Ccn1</em>) on chromosome 2BL</td>
<td>NW Europe, Australia, NW USA</td>
</tr>
<tr>
<td></td>
<td>Katyl</td>
<td>Con</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>Festiguay</td>
<td><em>Cre8</em> (formerly <em>CreF</em>) on chromosome 7L or 6B</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>AUS4930 = Iraq48</td>
<td>Possible identical genetic location as <em>Cre1</em> also resistant to <em>P. thornei</em></td>
<td>Under evaluation in Australia, France and CIMMYT</td>
</tr>
<tr>
<td></td>
<td>Molineux</td>
<td>Chromosome 1B</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>Raj MR1</td>
<td>One dominant gene</td>
<td>Released cv. in India</td>
</tr>
<tr>
<td><em>T. durum</em></td>
<td>Psathias 7654, 7655</td>
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<td>Not known</td>
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<tr>
<td><em>Triticosecale</em></td>
<td>T701-4-6</td>
<td><em>CreR</em> on chromosome 6RL</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>Drira</td>
<td>Not known</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>Ningadhu</td>
<td>Not known</td>
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<tr>
<td></td>
<td>Tahara</td>
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<td>Salvo</td>
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<tr>
<td><em>Secale cereale</em></td>
<td>R173 family</td>
<td><em>CreR</em> on chromosome 6RL</td>
<td>Australia</td>
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<tr>
<td><em>Aegilops tauschii</em></td>
<td>CPI 110813</td>
<td><em>Cre4</em> on chromosome 2DL</td>
<td>Australian synthetic hexaploid lines</td>
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<tr>
<td></td>
<td>AUS 18913</td>
<td><em>Cre3</em> on chromosome 2DL</td>
<td>Australian advanced breeding lines</td>
</tr>
<tr>
<td><em>A. peregrine</em></td>
<td>1</td>
<td><em>Cre</em> (3S) with <em>Rkn2</em> on chromosome 3S, <em>CRX</em> not yet located</td>
<td>Not known</td>
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<tr>
<td><em>A. longissima</em></td>
<td>18</td>
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</tr>
<tr>
<td><em>A. geniculate</em></td>
<td>79, MZ1, MZ61, MZ77, MZ124</td>
<td>Not known</td>
<td>France</td>
</tr>
<tr>
<td><em>A. triuancialis</em></td>
<td>TR-353</td>
<td><em>Cre7</em> (formerly <em>CreA et</em>)</td>
<td>France</td>
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<tr>
<td><em>A. ventricosa</em></td>
<td>VPM 1</td>
<td><em>Cre5</em> (formerly <em>CreX</em>) on chromosome 2AS</td>
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<td></td>
<td></td>
<td><em>Cre2</em> (formerly <em>CreX</em>) on genome N</td>
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