3 The need for integrated management of the cereal cyst nematodes, *Heterodera* spp. in Central Western Asia and North Africa

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Introduction

Bread wheat covers over 240 million hectares globally and is considered a staple food of around 40% of the global population and contributes one-third of total world grain production. It is projected that world wheat production must increase by at least 60% to meet the estimated wheat grain demand in 2050. Among biotic factors, plant parasitic nematodes, diseases and insects are important constraints leading to substantial reductions in per unit area production. Cereal cyst nematodes (CCN), root lesion nematodes and seed gall nematodes are significant species on wheat in most regions of the world and are present in the Central Western Asia and North Africa (CWANA) (Dababat and Fourie, 2018; Seid *et al.*, 2021). In the CWANA regions, wheat yield is negatively affected by a complex group of *Heterodera* species. *Heterodera avenae*, *H. filipjevi* and *H. latipons* are the most important CCN. The damage caused by CCN is not well known to those working in these countries.

This chapter emphasizes the economic importance, distribution, biology, symptoms of CCN in addition to recommended integrated nematode management (INM) tools to control CCN in wheat.

Economic importance

Wheat produced in the rainy winter season in the semi-arid regions of CWANA is highly vulnerable to CCN and considerable yield losses of up to 50% can occur (Dababat and Fourie, 2018). *H. avenae* is also the most destructive plant parasitic nematode of wheat in the climatic conditions of Northern Europe where it causes an estimated 10% loss in grain yield. Wheat in CWANA is a monoculture in most countries and CCN are serious problems with wheat grown in the cool rainy winter seasons across North Africa all the way to Northern Europe.

Although the true level of losses across all of CWANA is poorly worked out, the average yields in the region are very low when compared to the world average. This could be due to the lack of INM. Yield losses can exceed 50% under the harsh climatic condition characterized by low precipitation and high temperature in the region. Nevertheless, the reports regarding wheat grain yield losses do not accurately portray the magnitude of economic losses at the regional or national level because documentation has been mostly based on research plots located in infested areas of fields, i.e. sick plots (Smiley *et al.*, 2017).

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Further complications arise from reports initially attributed to yield reduction by *H. avenae* that are now identified as *H. filipjevi*, *H. latipons*, *H. australis*, or *H. sturhani* (Dababat and Fourie, 2018).

Host range

Heterodera avenae infects graminaceous crops especially wheat, oats, barley and rye. Other hosts include grasses from the genera *Agropyron*, *Alopecurus*, *Agrostis*, *Arrhenatherum*, *Anisantha*, *Brachypodium*, *Avena*, *Bromus*, *Dactylis*, *Echinochola*, *Festuca*, *Hordeum*, *Koeleria*, *Lolium*, *Lhalaris*, *Poa*, *Polypogon*, *Phleum*, *Setaria*, *Sorghum*, *Secale*, *Triticum*, *Trisetum*, *Vulpia*, *Zerna* and *Zea*. *Senebiera pinnatifida* belonging to the Brassicaceae family is the only non-graminaceous host recorded so far.

Major hosts of *H. filipjevi* include bread wheat, oat, false wheat, barley, rye and quack grass. However, *Triticum discocoides*, *T. durum*, *T. tauchi*, *T. monococcum*, *T. ovatum*, *T. turgidum*, *T. umbellatum*, and *T. ventricosum* are well characterized experimental hosts of *H. filipjevi*. Maize is a weak host of *H. filipjevi*. The J2 are able to invade maize roots but females fail to produce and therefore the crop is used in India as a trap crop.

Distribution

Heterodera avenae is the most widely distributed CCN around the globe (Fig. 3.1). Wheat producing regions with temperate climatic conditions in Asia, Africa, North and South America, Europe and the Mediterranean are typically CCN occurrence zones (Smiley *et al.*, 2017).

Symptoms of damage

CCN species establish their feeding sites called syncytia on the roots of wheat plants and this blockage of the vascular system leads to weakening of the root system. The damage caused is primarily characterized by patches of plants showing poor growth and chlorosis, which are unevenly distributed in the field. In a large field cultivated with a susceptible cultivar (monoculture is standard in the CWANA region), these patches merge and can cover the entire field within a few years. Severe infection of CCN in wheat leads to stunting, leaf chlorosis, reduction in leaf area, lower numbers of productive tillers and shorter spikes with fewer grains. Below ground symptoms caused by *H. avenae* include enhanced production of roots, i.e. root proliferation (Fig. 3.2A), and knot-like formations due to induction of syncytia containing multiple females (Fig. 3.2B). These root symptoms are usually noticeable 1 to 2 months after sowing in the CWANA region.

Biology and life cycle

Both *H. avenae* and *H. filipjevi* complete one life cycle in a year. In most cases they require a

Fig. 3.1. Distribution of *Heterodera avenae* around the globe. EPPO (2021) EPPO Global Database at https://gd.eppo.int.

Fig. 3.2. (A) Stunted wheat plants with knotted roots heavily infested with *Heterodera avenae* (left) compared with healthier plants (right). Photograph: Honglian Li, China. **(B)** White female of *Heterodera avenae* on wheat roots. Photograph: Zhenzhou, Henan, China.

diapause of up to 4 and 2 months, respectively, before the juveniles can emerge. The life cycle is illustrated in Fig. 3.3. Important is the fact that juvenile emergence only takes place when ample amounts of moisture under favourable soil temperatures are prevailing, which triggers the release of specific root exudates by the host plant. However, not all the J2 emerge at the same time, which is an important survival strategy harboured by CCN in the semi-arid regions.

Heterodera avenae infection in the root-tip region leads to growth inhibition, induction of typical branching and swelling of roots. Formation of syncytia differs between *H. avenae* and *H. latipons*; however, the impact of *H. avenae* on wheat growth and yield is more pronounced than *H. latipons*. This is due to different hatching behaviour of these two species and *H. latipons* juveniles penetrate at sites more distant from the root tip.

Race problem

CCN have considerable intraspecific diversity in the form of its pathotypes or biological races. [AU 1] The race system for CCN has been developed on the basis of the ability of the local populations to reproduce on barley cultivars containing different resistance genes according to ICCNTA (International Cereal Cyst Nematode Test Assortment). This system could be used to identify various races with distinct characteristics present in the CWANA region. The *H. filipjevi* pathotypes were tested for several populations in Turkey and fortunately were found to be similar. However, the race spectrum in the region for all CCN species needs to be collaboratively studied among the nematologists in the CWANA region so that different pathotypes can be identified and considered in the wheat-breeding programmes. The soil-borne pathogens programme at CIMMYT annually screens thousands of wheat lines and the most resistant lines are shared with the International Winter Wheat Improvement Program (IWWIP) who distribute those materials to more than 150 collaborators representing around 50 countries in the CWANA and beyond.

Interactions with other nematodes and pathogens

Although interactions among various plant pathogens are well established, such interactions regarding CCN are not well studied. This is the reason why only a few reports concerning complex interactions of CCN with other nematodes and pathogens are available in the literature. Cook in 1970 observed the first interaction of *H. avenae* with a fungal pathogen, *Gaeumannomyces graminis* var. *tritici*, the causal organism of take-all disease in wheat and described severe symptoms of take-all disease that are always associated with low population densities of *H. avenae* in the field. Smiley *et al*. (1994) further

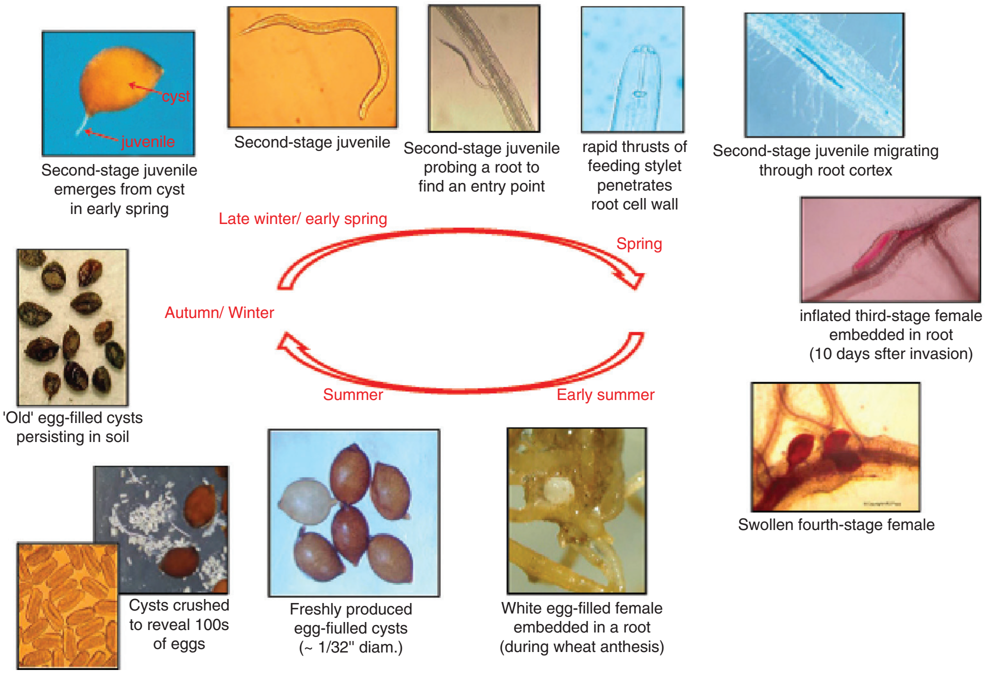

Fig. 3.3. Life cycle of *Heterodera avenae* (Courtesy of R. Smiley, Oregon, USA).

confirmed this interaction in winter wheat and reported substantial reduction in grain yield due to combined infestation of *H. avenae* and *G. graminis* var. *tritici* in the field.

Similar nematode–fungus interactions were reported for *H. filipjevi* and *Fusarium culmorum* pathogens in winter wheat in Iran under rainfed conditions. However, the effects of these two organisms on plant growth were additive rather than synergistic (Hajihassani *et al*., 2013).

Recommended integrated nematode management and optimization

Wheat growers in CWANA basically do not recognize nematodes as a problem. In fact, most of them do not know that what nematode species are in their fields affecting yield, which is why the term 'hidden enemy' perfectly applies to the problems in the region. INM is therefore not practiced in the entire region and nematode-induced yield losses are simply accepted.

The yield reduction in wheat due to CCN in CWANA could be lessened by improving and understanding the concept of INM in the region where the practice of winter monoculture of wheat is the norm. Management of cereal nematodes, especially CCN, could involve an integrated approach that includes crop rotation, genetic resistance, crop nutrition and appropriate water supply.

The following control measures are being used by some progressive wheat farmers in the CWANA region.

Crop rotation

Damage from CCN is greatest when monoculture practices exist especially with susceptible crops. Yield losses become very high in 2-year rotations of cereals even with the traditional summer fallow as well as in 3-year rotations such as winter wheat, spring cereal and a non-host broadleaf crop or fallow. Crop rotations that include broadleaf crops (tobacco), corn, fallow and resistant wheat, barley or oat cultivars reduce nematode density. For the most part, farmers in the CWANA region still perform summer fallow for two reasons, firstly to help the soil maintain high moisture levels and secondly to reduce diseases.

Weed control

CCN may also persist on a wide range of weed grasses. Grassy weeds such as quack grass, crabgrass, brome grass, foxtail, wild oat, rat-tail fescue and others should not be allowed to grow during any phase of a crop rotation in a field that is infested with CCN. Weed control is a commonly used practice in the CWANA – not to reduce nematode densities, but instead to keep the soil moisture content at higher levels to boost the establishment and growth of the next crop with ultimately better germination.

Resistance and tolerance of wheat and barley

The use of host resistance is an effective method of controlling CCN. Resistance is defined as the ability of the host to inhibit nematode multiplication. In some cases nematodes still penetrate resistant cultivars and cause damage even if not able to multiply. The benefit of resistance is that it reduces the intensity of risk to the next crop of barley, oats or wheat. Ideally, resistance should be combined with tolerance to nematode penetration.

The development of cultivars with only tolerance, which is the ability of the host plant to maintain its yield potential in the presence of nematodes could also be used in management where resistance is not known.

There are no wheat, barley or oat cultivars currently available that are fully resistant to CCN in CWANA.

Tolerance and resistance genes by CIMMYT for CCN were recently identified (Pariyar *et al.*, 2018; Dababat *et al.*, 2016). The most effective wheat resistance gene for controlling CCN and their pathotypes is Cre1 which has been crossed into local varieties. The Cre1 gene appears to suppress but not eliminate production of CCN. Sources of resistance for barley and oats have not yet been crossed into CWANA wheat varieties

Timing of planting and trap crops

Planting winter wheat rather than a spring crop of wheat, barley or oat cultivars can favour strong, deep root development before the majority of J2 emerge from cysts during the spring. In addition, where sufficient water is available, planting a susceptible host as a trap crop during the fall or early spring can reduce CCN densities in soil. The trap crop is invaded when J2 migrates from the cyst into the soil during early spring. The trap crop is then killed during mid-spring before new egg-bearing cysts can be developed. This strategy is particularly useful where growers plan to produce a warm-season crop such as chickpea or bean that can be planted during late spring after the trap crop has been killed in an infested field that will be planted to wheat or barley the following year.

Crop nutrition and water supply

Since the greatest crop loss occurs when nutrients or water are scarce at important growth stages, supplying optimal plant nutrition and, where possible, supplemental water during intervals of drought can minimize (mask) crop damage, particularly when the nematode damage is only slight or moderate. However, crops that are severely damaged by CCN usually do not respond well to additional applications of nutrients or water. If severe damage becomes evident early enough in the spring, it may be more profitable to destroy the crop and replace it with a non-host (broadleaf) crop.

Nematicides

Non-fumigant, in-crop nematicides especially the use of seed treatments is effective and widely used on other crops and has been used to reduce losses by CCN (Dababat *et al.*, 2014). However, the use of nematicides is not economically feasible on most grain crops.

Biological agents

Applications of currently available biological nematicides have not been effective for increasing the productivity of wheat in the region. However, in some locations, naturally occurring fungal or bacterial parasites invade and kill some of the CCN eggs that are still inside the cyst. These natural parasites of eggs reduce the density of CCN, but even in fields where they are known to be present and active, reduction in yields of wheat and barley continue to occur. Ways to amplify the benefit of these natural biological agents in commercial agriculture have not been identified.

Tillage

Tillage does not have an appreciable effect on the density of cereal nematode species. Populations are likely to be similar in both cultivated and till verses non-tilled fields.

Future research requirements

There is a need for more research to develop high-yielding and disease-resistant wheat cultivars adapted to a wide range of environments. In addition, improved technology for sustainable management of plant pathogens including CCN is needed (Ali *et al*., 2015). The major requirements for the future management of CCN are:

1. Create awareness among the farmers in the developing countries especially through educating researchers at the extension services to support growers.
2. Establishment of yield losses caused by a particular CCN species in real time, which could be challenging due to uneven distribution and presence of multiple species of CCN in a single field.
3. Molecular diagnostics for reliable identification of CCN species.
4. Because different CCN species behave differently to environmental and edaphic factors, the development of reliable epidemiological models would be helpful for the management of diverse CCN species
5. New chemistry nematicides, which are less dangerous for the environment and human health, must be developed and tested under the field conditions. Chemical companies should test any promising compound on a wide range of wheat germplasm having different levels of resistant to cereal nematodes to investigate whether chemicals have additive/synergetic effect on resistance behaviour or not?
6. Novel biological management options like seed treatment with biocontrol agents need to be examined further.
7. Cell phone apps based on the identification of the damage caused by CCN and other IT and social media services could be developed and utilized for better understanding for the farmers.
8. Understanding the interactions of CCN with other nematodes and plant pathogens is important to devise management approaches for multiple pathogens in a particular field.
9. There is a further need for the maintenance of genetic diversity in elite lines at CIMMYT so that national programmes can breed their own resistant cultivars adapted to the wide spectrum of CWANA environmental conditions.

Outlook: anticipating future developments

The accessibility to genomic resources like genome sequences and high throughput data on genomes and transcriptomes provides a huge array of information that could be used for wheat improvement for grain yield and CCN resistance. For instance, survey of complete genome sequences of different CCN may help to identify novel effector coding genes which could be manipulated through host-induced gene silencing technology. Likewise, recent release of complete wheat genome offers enormous opportunities to study and understand molecular wheat–nematode interactions leading to development of CCN-resistant wheat. Similarly, worldwide wheat germplasm collections of wild and cultivated wheat accessions provide unique opportunities for wheat genetic improvement. Furthermore, the availability of several genome-wide association studies could enable the wheat breeders to identify genomic regions associated with CCN resistance in wheat. Ali *et al.* (2019) recently reviewed and summarized the transgenic opportunities to enhance CCN resistance in wheat, which included employment of R-genes, host-induced gene silencing of vital effector CCN genes through RNAi, anti-feedant proteinase inhibitors, anti-invasion chemodisruptive peptides and manipulation of gene expression specifically in syncytia. One of the potential future directions to develop CCN resistance could be the pyramiding of two or more of these strategies which may lead to complete control of CCN in wheat.

Targeted mutagenesis through the application of CRISPR/Cas9 system could be deployed for non-transgenic and targeted deletion or addition of sequences in wheat genome for induction of CCN resistance in bread wheat.

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