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Incidence and geographical distribution of the cereal cyst nematode (CCN, *Heterodera* spp.) in winter wheat fields in Algeria

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Abstract

Incidence and geographical distribution of the cereal cyst nematode (CCN, Heterodera *spp.) in winter wheat fields in Algeria.* Cereal cyst nematodes (CCN, *Heterodera* spp.) are the most damaging plant–parasitic nematode species on wheat, causing severe economic loss in global wheat production. In summer 2015, we analyzed samples collected from 22 wheat fields in Algeria using the Fenwick can technique. The study revealed that 54.55 % of wheat fields were infested with cereal cyst nematodes. The species was observed in several locations in the northern part of Algeria but not in the southern desert area. Population densities of CCNs in soil varied between the regions at an infestation rate of between 0.6 ± 0.54 and 86.6 ± 19.96cysts/500 g of dried soil. Furthermore, we found an average of 56.33 ± 15.18 and 364.70 ± 81.93 second–stage juveniles and eggs per cyst. The infestation was most severe in cereal fields in Draa Semar and Djendel with 86.6 ± 19.96 cyst/500 g of soil and 57.4 ± 17.55 cysts/500 g of soil, respectively. Infestation was lowest in fields in Ras Elouad, Sidi Mbarek and Sedraia with 0.6 ± 0.54 cysts/500 g of soil; 1.6 ± 1.67 cysts/500 g of soil and 2.4 ± 1.67 cysts/500 g of soil, respectively. *Heterodera* spp. was distributed throughout the cereal growing province in Algeria and could cause economic loss in these regions.

Data published in Mendeley and Zenodo (Doi:10.17632/rtsbcn6k9y.1)

Key words: Cereal cyst nematodes, Incidence, Wheat, Distribution, Algeria

Resumen

Incidencia y distribución geográfica del nematodo enquistado de los cereales (CCN, Heterodera *spp.) en campos de trigo de invierno de Argelia.* Los nematodos enquistados de los cereales (CCN, *Heterodera* spp.) son los nematodos parásitos vegetales más dañinos para el trigo, especialmente en las zonas de cultivo de cereales, en todo el mundo. En el verano de 2015 tomamos muestras en 22 trigales y las analizamos utilizando la técnica del embudo de Fenwick. El estudio reveló que el 54,55 % de los trigales estaban infestados por nematodo enquistado de los cereales. La infestación se observó en varias localizaciones del norte de Argelia, pero no en la zona desértica del sur. Las densidades de población de CCN

en el suelo de las zonas infestadas variaron entre 0,6±0,54 y 8,6±19,96 quistes/500 g de suelo seco. Además, hallamos un promedio de 56,33±15,18 y 364,70±81,93 segundos estadios juveniles y huevos por quiste, respectivamente. La infestación era más severa en los campos dedicados al cereal de Draa Semar y Djendel, con 86,6±19,96 quistes/500 g de suelo y 57,4±17,55 quistes/500 g de suelo, respectivamente. Las tasas más bajas de infestación se observaron en los otros campos: Ras Elouad, Sidi Mbarek y Sedraia, con 0,6±0,54 quistes/500 g de suelo, 1,6±1,67 quistes/500 g de suelo y 2,4±1,67 quistes/500 g de suelo, respectivamente. *Heterodera* spp. estaba distribuido en toda la zona de cultivo de cereal de Argelia y podría causar una pérdida de rendimiento económico en esas regiones. Mostramos la distribución en un mapa.

Datos publicados en Mendeley y Zenodo (Doi:10.17632/rtsbcn6k9y.1)

Palabras clave: Nematodo enquistado de los cereales, Incidencia, Trigo, Distribución, Argelia

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Resum

Incidència i distribució geogràfica del nematode enquistat dels cereals (CCN, Heterodera *spp.) en camps de blat d'hivern d'Algèria.* Els nematodes enquistats dels cereals ((CCN, *Heterodera* spp.) són els nematodes paràsits vegetals més nocius per al blat, especialment a les zones de conreu de cereals, arreu del món. L'estiu de 2015 vam prendre mostres en 22 camps de blat i les vam analitzar mitjançant la tècnica de l'embut de Fenwick. L'estudi va revelar que el 54,55% dels camps de blat estaven infestats pel nematode enquistat dels cereals. La infestació es va observar en diverses localitzacions del nord d'Algèria, però no a la zona desèrtica del sud. Les densitats de població de CCN al sòl de les zones infestades van variar entre 0,6±0,54 i 8,6±19,96 quists/500 g de sòl sec. A més, vam trobar una mitjana de 56,33±15,18 i 364,70±81,93 segons estadis juvenils i ous per quist, respectivament. La infestació era més severa als camps dedicats al cereal de Draa Semar i Djendel, amb 86,6±19,96 quists/500 g de sòl i 57,4±17,55 quists/500 g de sòl, respectivament. Les taxes més baixes d'infestació es van observar als altres camps: Ras Elouad, Sidi Mbarek i Sedraia, amb 0,6±0,54 quists/500 g de sòl, 1,6±1,67 quists/500 g de sòl i 2,4±1,67 quists/500 g de sòl, respectivament. *Heterodera* spp. estava distribuït en tota la zona de conreu de cereal d'Algèria i podria causar una pèrdua de rendiment econòmic en aquestes regions. En mostrem la distribució en un mapa.

Dades publicades a Mendeley i Zenodo (Doi:10.17632/rtsbcn6k9y.1)

Paraules clau: Nematode enquistat dels cereals, Incidència, Blat, Distribució, Algèria

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Introduction

Wheat is the most important and most strategic cereal crop worldwide. In 2016, the world wheat production reached 759.7 million tons (Mt) (FAO, 2018). In Algeria, wheat was planted in 2 million hectares in the growing season of 2015–2016, with an estimated production of 3.3 Mt (FAO, 2017). This production does not meet Algeria's needs. In 2016/2017, Algeria imported 8 Mt of wheat (FAO, 2017). Among the biotic stresses, the obstacle to cereal production is the damage caused by plant–parasitic nematodes. The cereal cyst nematodes (CCNs: *Heterodera* spp.) are considered to have a major impact on decreasing the yield of cereals throughout the world (Hando, 2002; Namouchi–Kachouri and B'Chir, 2005; Akar et al., 2009; Toumi et al., 2013; Ahmadi and Tanha Maafi, 2014). Yield losses due to CCN on wheat are reported to be more than 50 % in Iran (Hadjihassani et al., 2010), 26–96 % in Tunisia (Namouchi–Kachouri et al., 2009), 24 % in Syria (Singh et al., 2009), 40–50 % in Morocco (Rammah, 1994 in Mokrini et al. [2018]), and between 23–50 % in Australia (Nicol et al., 2002). It has been calculated that the annual yield loss is 72 million Australian dollars in Australia (Brown, 1981 in Mokrini et al. [2018]). At least $US3.4 million in wheat production is estimated to be lost annually because of CCNs in the US states of Idaho, Oregon, and Washington (Turner and Subbotin, 2013).

Twelve out of 80 *Heterodera* species damage roots of cereals and grasses (Akar et al., 2009; Subbotin et al., 2010; Ahmadi and Tanha Maafi, 2014). Among these, *H. avenae* Wollenweber, 1924, *H. filipjevi* Madzhidov, 1981 and *H. latipons* Franklin, 1969 are the main species causing most serious damage to wheat (Nicol, 2002; Toktay et al., 2015; Tirchi et al., 2016).

Previous studies have indicated that CCN are potentially a limiting factor in wheat production in Algeria. The first record of a species from the *H. avenae* group on wheat in Algeria was reported by Scotto La Massese (1962). Since then, *Heterodera* spp. has become more dominant in northern Algeria in the cereal–producing regions, Tissemsilt (Djetti et al., 2014), Aïn Defla (Tirchi et al., 2016), Algiers (Haddadi et al., 2013), Tiaret (Mokabli et al., 2002; Haddadi and Mokabli, 2015). However, little is known about the occurrence and distribution of CCNs on wheat in Algeria.

The aims of the present study were: i) to investigate the infestation and distribution of CCNs (*Heterodera* spp.) collected from wheat fields in northern and southern of Algeria; ii) to evaluate the infestation levels and compare the obtained data with those from previous surveys carried out in Algeria; and iii) to draw a map showing the distribution of CCNs in the country.

Material and methods

Soil sampling

The northern region of Algeria is the most important area for growing cereals. After harvest in July and August of 2015, soil samples were taken from each field using a spade. The sites were situated in eight provinces (table 1).

Twenty–two soil samples were taken from fields in eight cereal growing provinces in Algeria. Each soil sample consisted of 13 soil cores or subsamples; a subtotal of 2.5 kg was collected from around the root systems at 15–20 cm deep after removing the top 5 cm layer from the rhizosphere of wheat plants. Subsamples were taken in a 'W' pattern through the fields. Samples were sent to the Laboratory of Nematology, High National School of Agronomy (E.N.S.A.) El Harrach Algiers, for further processing.

Nematode extraction and counting

Each soil sample was mixed thoroughly. Cysts were extracted from five subsamples of 500 g using the modified Fenwick can technique (Fenwick, 1940; Smiley, 2011) and full mature cysts retained on the 250–µm sieve were picked with a dissecting needle under a

Table 1. Geographical location of the study regions.
Tabla 1. Localización geográfica de las zonas de estudio.

Province	Area surveyed	Geographical location
Bouira	Aïn Bessam	36°18'01.3"N 3°41'03.0"E
	Bouira	36°22'02.5"N 3°51'57.1"E
Aïn Defla	Djendel Site 01	36°12'55.0"N 2°25'16.3"E
	Djendel Site 02	36°12'46.2"N 2°26'23.2"E
	Ain Soltane	36°13'30.9"N 2°19'59.2"E
	Aïn Defla	36°15'46.5"N 1°55'15.0"E
	Bir Ould Khelifa	36°10'05.8"N 2°13'33.7"E
Medea	Draa Semar	36°16'26.3"N 2°42'16.4"E
	Sedraia	36°15'02.9"N 3°29'09.5"E
Bordj Bou Arreridj	Medjana1	36°07'35.9"N 4°41'12.3"E
	Medjana2	36°06'56.0"N 4°42'44.6"E
	Sidi Mbarek Site 01	36°04'32.5"N 4°55'12.6"E
	Sidi Mbarek Site 02	36°05'58.9"N 4°53'07.9"E
	Ras Elouad	36°01'48.8"N 4°59'40.3"E
Tissemsilt	Tissemsilt	35°37'31.2"N 1°52'43.6"E
	Bni Maïda	35°33'01.0"N 1°50'33.2"E
	Ammari	35°33'40.0"N 1°38'50.0"E
	Dhaya	35°35'28.5"N 1°44'54.7"E
Mostaganem	Debdaba	35°54'04.9"N 0°07'47.5"E
Blida	Mozaya	36°28'09.2"N 2°39'31.1"E
Adrar	Zaouiet Kounta	27°12'57.2"N 0°11'53.9"E
	Timimoun	28°38'09.5"N 0°19'24.3"E

stereomicroscope. Species were identified based on morphological features (Handoo, 2002). Collected cysts were typically ovoid to lemon–shaped but had different sizes (Haddadi and Mokabli, 2015). Cysts were counted in 500 g of soil. The cysts were surface sterilized using sodium hypochlorite NaOCl (0.1%) for 5 min and rinsed several times with sterile distilled water for further studies. Cleaned and sterilized cysts were transferred into an Eppendorf and stored at 4 °C until use.

Population density of *Heterodera* spp. and data analysis

For each soil sample in the surveyed areas, the population density of CCNs is the number of cyst of *Heterodera* spp. in each 500 g of dry soil, and the number of eggs and second–stage juveniles per cyst. Eggs and J2s were obtained by crushing cysts. The means and standard deviations of the number of cysts, eggs, and J2s were calculated.

Data analysis

Data were analyzed using analysis of variance with a software R version 3.2.4. The average number of cysts was calculated for the five subsamples and expressed per 500 g of soil; the number of eggs and J2 in each cyst was estimated. Differences were tested using analysis of variance (ANOVA) followed by the Tukey HSD test to compare means if the *F*–value was significant. Differences were considered statistically significant if $P \leq 0.05$.

Results

Field infestation and distribution maps

Most study localities were situated in the northern part of Algeria. The results showed that 60 subsamples of 110 soil subsamples (54.55 %) were infected with *Heterodera* spp. (see dataset published at Mendeley, Doi: 10.17632/rtsbcn6k9y.3 and table 2).

CCNs were detected in almost all fields with monoculture of wheat in seven provinces in Bouira (Aïn Bessam and Bouira), Aïn Defla (Djendel Site 01, Djendel Site 02 and Aïn Defla), Medea (Draa Semar, Sedraia), Bordj Bou Arreridj (Sidi Mbarek site 1, Sidi Mbarek site 2 and Ras Elouad), Tissemsilt (Bni Maïda), Mostaganem (Debdaba) and Blida (Mozaya). *Heterodera* spp. was not detected in nine provinces *i.e.* Ain Soltane, Bir Ould Khelifa, Medjana Site 1, Medjana Site 2, Tissemsilt, Ammari, Dhaya, Zaouiet Kounta, and Timimoun.

Both fields in Bouira province were infested. In these areas, crops rotate annually with other vegetables like potatoes. In Aïn Bessam, we observed that CCNs were less spread than previously reported (Haddadi and Mokabli, 2015). The average numbers of cysts, eggs and juveniles/100 g of soil in wheat fields was higher than in barley fields (Ahmed and Tanha Maafi, 2014). The fields were cultivated with cereal; according to Smiley et al. (2007), *Heterodera* spp. attack Poaceae like wheat and barley.

In Aïn Defla, 25 soil subsamples were analyzed in five fields and three samples were positive. The high infestation was counted in Djendel Site 02 with a monoculture of winter wheat. No infestation was found in Ain Soltane with a fallow period or in Bir Ould Khelifa where cereal was rotated with potato. The impact of this parasite depends on the host (Ahmadi and Tanha Maafi, 2014).

The province of Medea was represented by two fields, Draa Semar and Sedraia, where there was a monoculture of wheat. The average number of cysts was high in both fields. CCNs are highly developed and associated with yield loss in semi–arid regions (Sikora, 1987).

In Bordj Bou Arreridj, CCNs were found in 60 % of fields (25 subsamples). In Medjana there were no CCNs because fields rotated each year with other crops like oat or were fallow crops. However, a few cysts were found in Ras Elouad, a field some distance away from the other field cited above. The lowest level of cysts, eggs, and juveniles was recorded in Sidi Mbarek Site 1 and Ras Elouad. This is probably due to the practice of fallow each second year. Fallow reduces the density population of CCNs in soil by up to 70 % (Singh et al., 2009).

CCNs were detected in only one (Bni Maïda) of the four fields in Tissemsilt. The results are in agreement with our study in 2013 reported by Djetti et al. (2014) in the same field. In addition, the fields Dhaya and Ammari showed zero infestation which is probably related to the cereal/legume rotation practiced in these localities. This absence of CCNs does not necessarily mean that the fields are free of these parasites (Haddadi and Mokabli, 2015).

All the subsamples in the field of Debdaba in Mostaganem in north–west Algeria were infected with CCNs. In the west of Algeria, in Sidi Bel Abbes, cyst extraction from soils taken from the Lamtar and Telagh fields cultivated on wheat showed 9 and 11 cysts/100 cm^3 of soil, respectively (Smaha et al., 2018a), In Mascara (El Ghoumri), a field of barley crops, an average of 21.7 cysts/kg was found (Haddadi and Mokabli, 2015).

The area of Mozaya (Blida) was cultivated by winter wheat in 2015. It had previously been

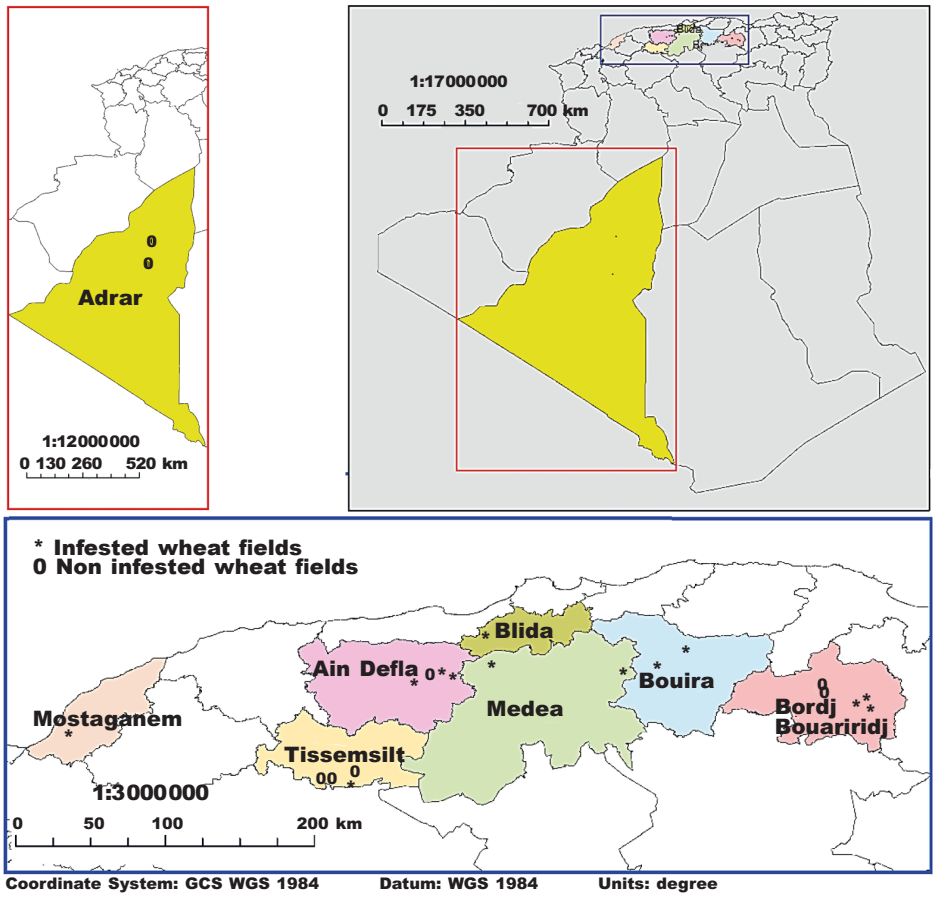

Fig. 1. Distribution map of cereal cyst nematodes, *Heterodera* spp., in Algeria 2015–2016.
Fig. 1. Mapa de distribución de nematodos productores del quiste de los cereales Heterodera *spp., en Argelia en los años 2015–2016.*

rotated with potato and fallow. The rotation with another non–host crop is the best method to control the parasite (Rivoal and Bourdon, 2005). Singh et al. (2009) in Mokrini et al. (2017) showed that the nematode densities decreased after rotation with non–host crops like carrot (*Daucus carota*), fenugreek (*Trigonella foenum–graecum*), and onion (*Allium cepa*), or by fallow and summer ploughing.

The Sahara fields Zaouiet Kounta and Timimoun are now cultivated for the first time with cereal crops. In these two areas, there was a pivot irrigation system and each year they changed the pivot to other places. There were no CCN in these areas. They have not been detected in the southern Algerian irrigated areas near Adrar (Haddadi, 1999 in Haddadi et al. [2013]). In Jordan, the Mediterranean cereal cyst nematode is widely distributed in several locations in the northern and southern Mediterranean and the eastern desert areas, with an incidence that varies from 30 to 100 % (Al Abed et al., 2004). In Egypt, CCN were found in five of seven regions in the Ismailia governorate with an occurrence of 79.4 %. The population density was 29 cysts on average, 10 J2s/100 cm^3 (Baklawa et al., 2015). The

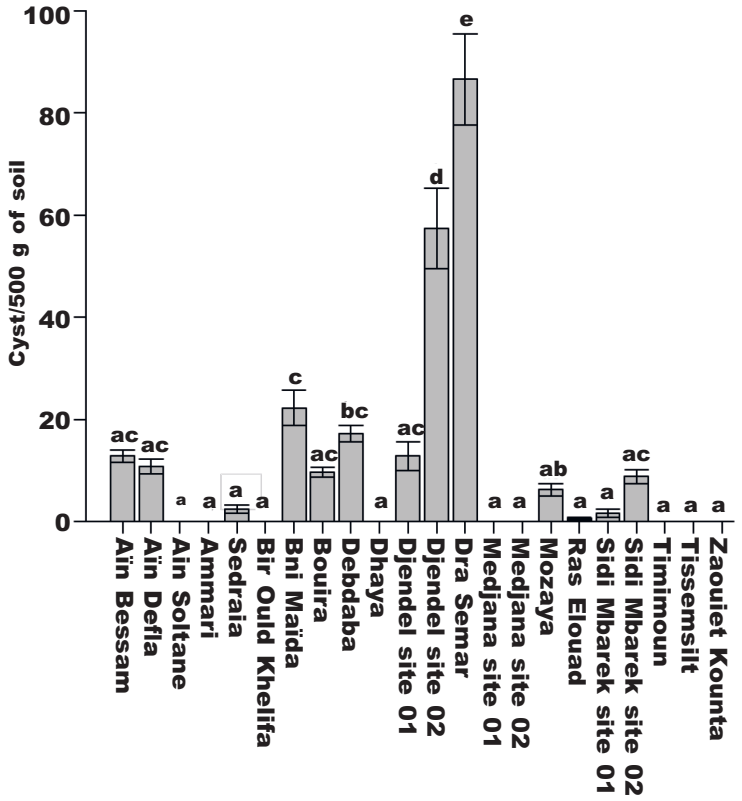

Fig. 2. Analysis of variance of cysts/500 g of soil in the surveyed localities.
Fig. 2. Análisis de variancia de quistes/500 g de suelo en las localidades de estudio.

survey of CCNs showed that 56 % of wheat fields in the East Anatolia Region of Turkey were infested. Cyst nematodes were detected in all fields with wheat in Elazig, Malatya, Erzurum, Erzincan, Sivas, Kars and Igdir provinces (Toktay et al., 2015). According to Toumi et al. (2015) a survey conducted in north–eastern Syria in wheat and barley growing areas revealed that 62 % of the fields were infested with the Heterodera species. In Russia (Chelyabinsk Region), the highest nematode population density was found between 2010 and 2012, with a mean density of 100 cysts/100 g soil (Pridannikov et al., 2015).

The present survey showed that 54.55 % of wheat fields in Algeria were infested. However, Heterodera spp. was not detected in the Sahara of Adrar. Infestation levels were highest in Medea. In contrast, the lowest levels of infestation were recorded in Bordj Bou Arreridj. *Heterodera* spp. was detected in 13 geographical areas. Figure 1 shows a distribution map of CCNs in wheat fields of Algerian provinces.

The analysis of variance (ANOVA)

Cysts/500 g of soil

Our results using analysis of variance (ANOVA) showed a significant difference between infestations with CCNs (cysts/500 g of soil) and regions with a probability of 2e–16 < 0.001. In addition, for the groups analyzed by Tukey test, from seven groups, group 'd' and group

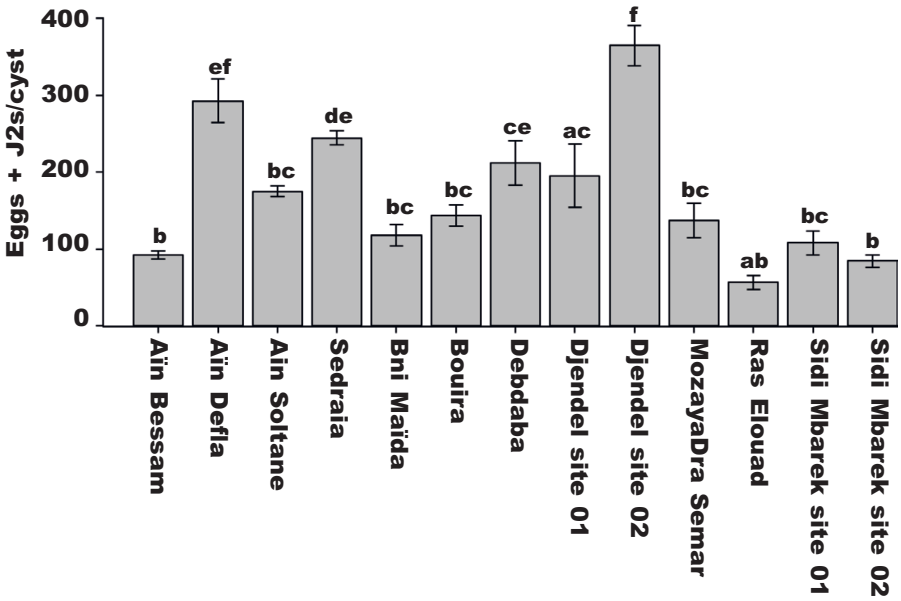

Fig. 3. Analysis of variance of eggs + J2s/cyst in each infested field.
Fig. 3. Análisis de variancia de huevos + J2/quiste en cada campo infestado.

'e' (Djendel Site 02 and Draa Semar) had the highest level of infestation. The lowest density of Heterodera spp. was found in–group 'a'. Densities in the other groups are shown in figure 2. Several authors have reported a high occurrence and distribution of these CCNs in Algeria (Mokabli et al., 2002; Djetti et al., 2014; Smaha, 2014; Haddadi and Mokabli, 2015).

Eggs +J2s/cyst

Thirteen of the 22 fields were infested. The statistical analysis indicated a probability of f value < 0.001. For the Tukey test there were nine groups: the 'bc' group contained Bouira, Debdaba, Mozaya, and Sidi Mbarek site1. Figure 3 shows all groups.

Discussion

Cereal cyst nematodes (CCNs) are the most important pathogens of cereal crops in many parts of the world, with particularly adverse efects on the production of wheat and barley. Three species of CCN belonging to the *Heterodera avenae* group have been identified in cereal fields. *H. avenae* is widely distributed in temperate wheat–producing regions worldwide (Nicol et al., 2003). In Sicily, Italy, *Heterodera latipons* and *Heterodera hordecalis* appear to be the most common species in durum wheat (*Triticum durum*) and barley (*Hordeum vulgare*) samples (Lombardo et al., 2009). In Algeria, Tirchi et al. (2016), identified *H. avenae* in four localities in Ain Defla based on morphological characters and molecular identification. Smaha et al. (2018a, 2018b) also determined that *H. filipjevi* and *H. hordecalis* was distributed in wheat growing areas. In Mascara, the Mediterranean cereal cyst nematode *H. latipons* was reported by Mokabli et al. (2002). In the present study, after studying winter wheat growth in 22 fields in eight provinces, we found that 13 fields were infested by *Heterodera* spp. The degree of infestation varied. This survey shows that the major cereal growing areas in Algeria

are commonly infested by *Heterodera* spp.

CCNs can seriously reduce yields of cereal crops, especially in temperate and semi–arid regions (Rivoal and Cook, 1993; Nicol and Rivoal, 2008). To keep the population densities of this parasite below damage levels, appropriate management processes, such as crop rotation, are necessary (Mokabli et al., 2002; Smiley et al., 2007), Crop rotations that include broadleaf crops, corn, fallow, and resistant wheat (Toumi et al., 2015), barley, or oat varieties can greatly reduce the nematode density. In addition, growing susceptible hosts less than 50 % of the time in heavy–textured soils and less than 25 % of the time in light–textured soils could dramatically reduce CCN (Yan and Smiley, 2010). Furthermore, studies about the morphological characters as well as morphometrical and molecular analysis should be conducted to identify and separate species of the Heterodera genus. In Algeria, regional and intensive surveys are necessary to further understand the geographical distribution of this parasite and to investigate their relation with different hosts.

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