

## RESEARCH PAPERS

# *Heterodera latipons* on barley in Jordan

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**Summary.** A survey of major barley-growing areas in Jordan was conducted to determine the prevalence and degree of infestation of the Mediterranean cereal cyst nematode (MCCN) *Heterodera latipons*. The nematode was found in several locations in the northern and southern Mediterranean and the eastern desert areas of Jordan, but it was not detected in the northern Jordan valley and the southern desert areas. In those areas where the cyst nematode occurred, the incidence varied between areas from 30 to 100%. The degree of infestation varied from moderate to very severe. Infestation was most severe in the fields of the Northern Mediterranean area. The MCCN completed one life cycle per growing season. Studies on three isolates of the nematode from three areas, Ar-Ramtha, Madaba, and Al-Karak, revealed that the morphometrics varied little among these isolates. These three isolates as a whole varied in their virulence to two commonly used barley cultivars, Rum and Acsade 106, being more virulent on cv. Rum than on cv. Acsade 106. However, the isolates also varied among each other in their virulence to the same cultivar, Ar-Ramtha being the most virulent on cv. Rum, Madaba the most virulent on cv. Acsade 106.

## Introduction

In Jordan, barley (*Hordeum vulgare*) is grown in relatively low rainfall areas. A total of 57,500 ha were planted with barley in the growing season of 1999–2000, with an estimated production of 12,000 tons of grains (Anonymous, 2000). This production is low and does not satisfy Jordan's needs. In 2000, Jordan imported 600,000 tons of barley for livestock feed (Anonymous, 2000). There is therefore a need to increase barley production in order to reduce imports, and also incidentally to increase animal production. However, one of the

obstacles to barley production is the damage caused by plant parasitic nematodes. The cereal cyst nematode *Heterodera avenae* and the Mediterranean cereal cyst nematode (MCCN) *H. latipons* are the most common and important species of cyst nematodes on barley (Meagher, 1977; Sikora, 1988; Philis, 1997). The MCCN is a parasite of several cereal crops, such as wheat, barley and oat in several Mediterranean countries: Cyprus, Israel, Italy, Jordan, Libya, Spain, and Turkey (Franklin, 1969; Cohn and Ausher, 1973; Mor *et al.*, 1992; Yousef and Jacob, 1994; Philis, 1995; Rumpfenhorst *et al.*, 1996).

In the Jordan Valley the MCCN was first detected in two wheat fields at low population densities (Yousef and Jacob, 1994); on barley it was first noted in the in the Ar-Ramtha region of the Northern Mediterranean area in 1996 (Abu Gharbieh and

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Al-Banna, unpublished data). The discovery of the MCCN on barley led to the present survey, whose aim was to determine the distribution and degree of infestation of *H. latipons* in the major barley-growing areas of Jordan. The pathogenicity of three geographical isolates on two commonly used barley cultivars was also assessed.

## Materials and methods

### Field surveys

A survey of 52 barley fields in Jordan located in five phytogeographical areas was conducted in July and August 1999, after the harvest (Table 1). Fields that harboured *H. latipons* were further sampled during the same growing season in November 1999, and in March and June 2000 to study the life cycle of the target cyst nematode. Soil and root samples were collected in a "W" pattern through the fields. Each soil sample consisted of 20 soil cores, each with 200 g soil collected from around the root systems.

### Nematode extraction, fixation, mounting, and identification

Each soil sample collected after harvest was mixed thoroughly, and three subsamples of 100 g each were air-dried and the cysts extracted using the flotation method (Southey, 1986). The cysts were counted and the results averaged (Table 1). Vulval cones were prepared as described by Helling (1965). Second-stage juveniles (J2s) were obtained from cysts, while males were extracted from 100 g soil samples using the Baermann funnel technique (Schindler, 1961). Males and J2s were killed and fixed in hot buffered formalin (Humason, 1972). Permanent mounts of the different developmental stages were prepared following Seinhorst (1959). Morphological characteristics of cysts, J2s, and males were used to identify the species using the original description and a diagnostic key (Franklin, 1969; Mulvey and Golden, 1983).

To study the life cycle of the target nematode, soil and root samples were collected at different times during the growing season. Soil samples were

Table 1. Occurrence and degree of infestation of *Heterodera latipons* in the major barley producing regions, in Jordan, during 1999–2000.

Phytogeographical region	Fields (No.)	Incidence (%)	No. of cysts /100 g soil	No. J2s + eggs /100 g soil	Degree of infestation <sup>a</sup>
Northern Mediterranean					
Ar-Ramtha	10	100	9–44	600–3800	Severe-very severe
Irbid	3	67	9–13	750–1100	Severe-very severe
Madaba and Amman	7	86	8–37	450–3200	Medium-very severe
Jarash	2	50	17	700	Severe
Southern Mediterranean					
Al-Karak	7	57	4–12	336–1500	Medium-very severe
At-Tafilah	2	50	5	400	Medium
Ash-Shawbakbak	3	67	4–8	455–500	Medium
Northern Jordan Valley					
Karameh	2	0	0	0	Undetectable
Dier Allah	3	0	0	0	Undetectable
Eastern Desert					
Mafraq	10	30	5–18	446–600	Severe
Southern Desert					
Al-Mudawwarah	3	0	0	0	Undetectable

<sup>a</sup> Very low, J2 and eggs/100g soil <100; low, 100–300; medium, 301–500; severe, 501–1000; very severe, >1000 (Farivar-Mehin and Shakeri, 1994).

processed as described above, to detect cysts, J2s, and males of *H. latipons*. Barley roots were stained with acid fuchsin and examined for the occurrence of the different developmental stages of the nematode.

#### Estimation of the population densities

Population densities were expressed as number of eggs and J2s per 100 g air-dried soil. The eggs and J2s were extracted from the cysts by placing the cysts in a beaker and crushing in 20 ml of tap water. The infestation scale developed by Farivar-Mehin and Shakeri (1994) was followed.

#### Virulence of three geographical isolates of *H. latipons* on two barley cultivars

Isolates obtained from three naturally infested barley fields in the Ar-Ramtha, Madaba, and Al-Karak production areas were first identified as to species. Identification was based on the morphological and morphometrical characters of J2s, males, cysts and eggs. J2s were obtained from cysts placed in water. Males were extracted from soil by a modified Baermann funnel (Schlinder, 1961) or directly by dissecting the roots after staining with acid fuchsin. Gravid females (white cysts) were collected by a flotation method, examined and measured in water. Vulval cones were prepared as described by Hesling (1965). Eggs were extracted directly from cysts and mounted in water.

The three selected isolates were tested for pathogenicity on two common barley cultivars, Rum and Acsade 106. In the summer of 2000, dry soil samples were randomly collected at a depth of 10–30 cm from three *H. latipons* infested fields at locations in Ar-Ramtha, Madaba, and Al-Karak. Naturally infested soil with 2 eggs g<sup>-1</sup> soil was used in this test. Populations with higher densities were diluted with sand. Therefore, the initial population (Pi) was 300 eggs + J2s.

Seeds of barley (cv. Acsade 106 and Rum) were sown in 7-cm-diameter plastic pots (4 seeds per pot) filled with 150 g naturally infested soil. Before sowing, the pots were moistened to field capacity for 20 days and maintained at a constant temperature (10°C). The pots were then moved to a temperature-controlled growth chamber (16°C) and arranged in a completely randomized design with five replicates. Plants were removed from the soil ten weeks after sowing, and the final populations of

eggs and J2s (Pf) were recorded. The final population was calculated by placing and crushing the cysts, collected from each pot, in 20 ml of tap water. The egg suspension was then stirred on a magnetic stirrer and two 1 ml samples were removed to count eggs and J2s. The reproduction factors Rf (Pf/Pi) were also calculated. Data were subjected to analysis of variance (ANOVA), and means were separated by Fisher's protected LSD test (Cochran and Cox, 1957).

## Results

*Heterodera latipons* was detected in 66% of the barley fields sampled in the three phytogeographical areas: Northern Mediterranean, Southern Mediterranean and Eastern Desert, but it was not found in the Northern Jordan Valley or in the Southern Desert area (Table 1). The incidence of the cyst nematode in the two Mediterranean areas ranged from 50% in Jarash and At-Tafilah, to 100% in Ar-Ramtha (Table 1). In the Eastern Desert the MCCN was found in only 30% of fields sampled, but infestation was severe.

The degree of infestation of the MCCN on barley varied among and within the three phytogeographical areas (Table 1). The greatest number of severely infested fields was in the Northern Mediterranean area.

*Heterodera latipons* completed only one cycle in all areas during the growing season studied. The first barley root and soil samples taken in November, and coinciding with plant emergence at all locations, revealed the occurrence of J2s. Root samples collected in March showed females, white cysts, and males. J2s were not recovered from the soil samples at this sampling date. At the end of the season in June and after harvest, only brown cysts were found in the root and soil samples.

#### Virulence of the three geographical isolates of *H. latipons* on two barley cultivars

The three geographical isolates had morphological features of the different developmental stages of various *H. latipons* in common. The gravid female was white, with a lemon-shaped body, and a white sub-crystalline layer that was sloughed off as the female transformed to a cyst. A small egg sac was sometimes observed with no eggs. The vulval cone with semi-fenestrae was separated by

a distance greater than the fenestral width and the vulval slit was short. A strong underbridge was present and bullae were absent.

Males were characterized by a cylindrical body, slightly twisted near the spicule, offset heads, strong stylets, dorsal esophageal glands open 3–5  $\mu\text{m}$  behind the stylet base, a lateral field marked by four lines, and a bow-shaped spicule. J2s had a cylindrical body, curved dorso-ventrally when killed by heat, and a well developed stylet with anteriorly concave knobs.

Cysts from Madaba were larger (length–width) 578–455  $\mu\text{m}$  than cysts from Ar-Ramtha 557–438  $\mu\text{m}$  and Al Karak 549–423  $\mu\text{m}$  (Table 2). The greatest female body length was likewise found in an isolate from Madaba. The vulval cone measurements were similar among the three isolates. The Al Karak isolate had the greatest fenestral and semifenestral length but the shortest underbridge

length. There were slight differences between the three isolates in egg measurements (Table 2).

Male measurements varied only slightly among isolates. Males from Madaba were larger and with longer stylets than the other two isolates. Males of the Ar-Ramtha isolate had the shortest spicules (Table 4).

The J2s recovered from the cysts collected in Madaba were the longest and the distance from the anterior end to the excretory pore was greatest. The J2s of the Al Karak isolate had the longest tail and the shortest distance from the anterior end to the start of overlapping (Table 3).

The three geographical isolates of *H. latipons* reproduced well on the two cultivars (*Rf* from 4.58 to 6.2 [Table 5]). These isolates were more virulent on cv. Rum than on cv. Acsade 106. The highest *Pf* (cysts and eggs) and *Rf* values were found in cv. Rum (Table 5). On the other hand,

Table 2. Morphometric data ( $\mu\text{m}$  or ratio) of cysts, females, eggs and vulval cones (n=10) of three *Heterodera latipons* isolates.

Stage/structure and character	Ar-Ramtha				Madaba				Al-Karak			
	Mean	Range		SD	Mean	Range		SD	Mean	Range		SD
Cyst												
Cyst length without neck (L)	557	497	656	54	578	476	731	80	549	462	656	57
Cyst width (W)	438	366	546	53.5	455	338	614	90.1	423	359	483	30
L/W ratio	1.2	1.1	1.5	0.1	1.3	1.1	1.4	0.1	1.3	1.2	1.5	0.1
Female												
Body length without neck (L)	509	380	621	76	514	359	628	86	489	376	628	88
Body width (W)	393	276	496	75	412	269	511	76	379	241	490	82
L/W ratio	1.3	1.1	1.5	0.1	1.2	1.1	1.3	0.1	1.3	1.2	1.6	0.1
Neck length	86	62	104	16.3	80	55	97	12.8	87	69	97	8.3
Stylet length	26.2	23.3	28.6	1.8	25.9	21.4	28.6	2.1	26.1	23.2	28.6	1.7
Egg												
Length (L)	115	110	124	5.8	115	110	124	5.6	115	113	124	4.6
Width (W)	47	41	48	3.6	47	41	48	3.7	48	45	51	2.4
L/W ratio	2.7	2.2	3	0.3	2.7	2.3	3	0.3	2.7	2.4	2.9	0.2
Vulval cone												
Fenestral length	63.5	53.6	75.9	7	63.6	55.3	71.4	4.9	66	53.6	74.9	7.6
Fenestral width	21.9	17.3	28.6	3.4	20.1	14.2	28.6	3.6	21.1	17.9	23.2	2
Semifenestral length	16.1	12.5	21.4	2.8	15.9	12.4	21.4	2.5	16.6	14.3	21.4	2.2
Vulval slit length	7.9	5.4	10.7	1.5	6.9	5.4	8.9	1.3	7.2	5.3	10.7	1.8
Width of vulval bridge	30.3	21.4	35.7	4.6	30.3	21.4	35.7	4	30.5	19.6	42.8	6.7
Underbridge length	105	71.4	135.6	21.6	107.4	85.6	121.4	11	101.6	78.5	121.4	15.1
Underbridge width	10.6	7.14	14.3	2.4	11.9	8.9	14.2	1.9	11.6	8.9	14.2	1.5

Table 3. Morphometric data ( $\mu\text{m}$  or ratio) of second-stage juveniles (n=10) of three *Heterodera latipons* isolates.

Character	Ar-Ramtha			Madaba			Al-Karak		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Body length (L)	513	469-538	20	533	480-587	32	518	483-559	23
Midbody width (W)	22	21-23.6	0.8	22.3	21-23.6	1	23.8	21-26.3	1.6
L/W	23.2	21.3-25.6	1.5	23.9	22.8-25.9	1	22.0	19.7-24.3	1.2
Distance from anterior end to median bulb	77.2	71.8-82.3	3.6	77.4	73.5-82.3	2.7	76	66.5-82.3	4.1
L/distance from lip to median bulb	6.6	5.7-7.5	0.5	6.9	6.1-7.6	0.5	6.8	6.6-8	0.4
Tail length	53.5	47.3-59.5	3.8	55	49-60.4	4.5	57	49-67.8	5.7
L/tail length	9.6	8.8-11	0.7	9.7	9.1-10.5	0.4	9.2	8.0-10.7	0.9
Body width at anus	15.5	14.9-16.6	0.7	15.8	13.1-17.5	1.4	15.9	14-17.5	1.3
Tail length/anal body width	3.4	3-4	0.3	3.5	3.1-3.8	0.3	3.6	3.1-4.2	0.3
Length of hyaline tail tip	30.8	28-35	2.8	31.6	27.1-36.8	3.1	28.2	24.5-31.5	2.3
Stylet length	26.4	23.3-29.4	5.9	26.5	25.4-28.9	1	25.6	23.6-28	1.3
Hyaline tail tip/stylet length	1.2	0.8-1.5	0.2	1.2	1.1-1.4	0.1	1.1	0.9-1.2	0.1
Distance from dorsal gland opening to stylet base	4.9	4.4-6.1	0.6	4.8	4.3-5.2	0.5	4.8	4.4-5.3	0.5
Head width	9.6	8.8-10.5	0.7	10.2	8.8-11.4	0.7	9.2	8.8-10.5	0.6
Head height	5.1	3.5-6.1	0.8	6.3	5.3-7.9	0.9	4.5	3.5-5.3	0.5
Distance from anterior end to start of overlapping	120.2	106.8-138.3	10.2	121.6	115.5-127.8	4.6	116.4	103.3-134.8	11.2
Distance from anterior end to end of overlapping	167.1	147-192.5	11.5	166.4	155.8-173.3	5.1	165.3	147-173.3	7.7
Distance from anterior end to excretory pore	110.6	101.5-117.3	4.5	112.1	105.9-122.5	5.7	107.1	94.5-117.3	6.8

Table 4. Morphometric data ( $\mu\text{m}$  or ratio) of males (n=10) of three *Heterodera latipons* isolates.

Character	Ar-Ramtha			Madaba			Al-Karak		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Length (L)	1202	1070-1346	89	1224	1001-1449	132	1178	966-1380	123
Width (W)	28.9	24.5-32.4	2.3	29.6	25-32.1	2.1	29.6	25-35.7	3.6
L/W	41.7	35.4-45.6	3.5	41.3	35.2-47.8	3.9	40.1	34.4-48.3	3.9
Esophagus length	119.5	103.3-133	9.6	115.3	106.8-127.8	6.6	119.3	113.8-124.9	4.4
L/esophagus length	10.1	8.5-11.3	0.8	10.6	9-12.2	0.9	9.9	8.2-11.7	1.1
Stylet length	27.8	24.5-31.5	2.3	29.1	27.1-30.6	1.1	28.4	24.5-30.6	2.2
Stylet knobs width	5.4	4.4-7	1.0	4.6	4.4-5.3	0.4	5.2	3.5-6.1	0.9
Stylet knobs height	3	2.6-3.5	0.5	2.2	1.8-2.6	0.5	2.3	1.8-2.6	0.7
Head height	5.7	5.3-7	0.6	6	5.3-7	0.6	5.4	4.4-6.1	0.7
Head width	11.3	8.8-13.1	1.3	11.2	10.5-12.3	0.7	11.2	10.5-12.3	0.7
Distance from dorsal gland opening to stylet base	4	2.6-5.3	0.9	3.9	2.6-4.4	0.6	4.3	3.5-5.3	0.7
Spicule length	33.5	30.6-36.8	2.3	35.3	31.5-38.5	2.3	35.4	33.3-38.5	1.6

Table 5. Reproduction of three geographical isolates of *Heterodera latipons* on barley cv. Rum and Acsade 106.

Isolate	Cultivar	No. of cysts/pot	$Pf^{a}$ (eggs+J2)	Reproduction factor <sup>b</sup>
Ar-Ramtha	Rum	14.2 a <sup>c</sup>	1862 a	6.20 a
	Acsade106	10.8 c	1359 c	4.58 c
Madaba	Rum	13.8 ab	1749 ab	5.78 ab
	Acsade106	11.4 bc	1538 abc	5.14 abc
Al-Karak	Rum	12.8 abc	1721 ab	5.66 abc
	Acsade106	10.6 c	1401 bc	4.76 bc
LSD at $P=0.05$		2.43	353	1.17

<sup>a</sup>  $Pf$  (final population), No. of eggs+J2s/pot (150 g soil) at the end of the experiment.

<sup>b</sup> Reproduction factor,  $Pf/Pi$ , where  $Pi$  (initial population) at the beginning of the experiment was 300 eggs + J2s/pot.

<sup>c</sup> Values are the means of five replicates. Means in a column followed by the same letter are not significantly different ( $P<0.05$ ) according to Fisher's protected LSD.

the Ar-Ramtha isolate was more virulent on cv. Rum than the other two isolates. The  $Pf$  (cysts and eggs) and  $Rf$  values of cv. Rum infested with the Ar-Ramtha isolate were higher than those of the other two isolates, while the Madaba isolate was more virulent on Acsade 106 than the other two isolates with higher  $Pf$  and  $Rf$  values (Table 5).

## Discussion

*Heterodera latipons* is widely distributed through the major barley growing areas in the two phytogeographical Mediterranean regions of Jordan (Table 1). There were variations in both *H. latipons* prevalence and the degree of infestation among the studied areas. Variations in the levels of infestation can probably be attributed to environmental factors and agricultural practices. In the Ar-Ramtha area, which had the highest level of infestation, the soil type was classified as clay loam (31% clay, 43% silt, and 25.6% sand). In Cyprus, where yield losses caused by this nematode reached about 50% (Philis, 1988), the soil is also classified as clay loam. The MCCN also exhibited a high level of infestation in Madaba, which has clay soils, and in Al-Karak, with a silty clay loam soil. The data indicate that *H. latipons* is found in a wide range of soil types.

The severe infestation in several barley growing areas of Jordan can also be due to monoculturing and continuous growing of the same susceptible barley cultivar. Philis (1997) similarly found that the MCCN was particularly widespread in those

areas of Cyprus where barley had been grown in monoculture for many years. This author also reported that where drought and monoculturing prevailed, cyst nematode infestation was more severe.

The MCCN was not detected in samples from the Northern Jordan Valley and this may be explained by the higher soil temperatures in that area, where the average monthly temperatures in November and December were 24 and 19°C respectively. According to Al-Abed (2001) high temperatures (>20°C) decreased the rates of hatching, penetration and development of *H. latipons*. Yousef and Jacob (1994) first reported this cyst nematode in two wheat fields in the Jordan Valley though population densities were low, probably because conditions for the nematode were unfavorable.

Although the soil type in Al-Mudawwarah (>80% sand) is optimal for nematode reproduction and development (Al-Abed, 2001), none of the fields in this area were infested with MCCN. The absence of the nematode here might be due to the fact that the area surveyed was developed for agriculture only recently and that barley production is not regular: farmers mainly rotate this crop with vegetables, especially potato.

*Heterodera latipons* completed only one generation per year which confirms the findings results previously reported (Mor *et al.*, 1992; Philis, 1999).

The data from the morphometric studies and the pathogenicity test on the two barley cultivars revealed some variation among the three isolates. These variations in J2, male, female, and cyst morphometrics were related to environmental and nutritional factors (O'Brien and Fisher, 1979).

The pathogenicity test indicated that the two commonly used barley cultivars Rum and Ac-sade 106 were susceptible to the three isolates with minor variations in their response. In order to evaluate the genetic diversity among Jordan populations of *H. latipons*, a race determination test should be conducted as well as molecular analysis.

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