The Effect of Initial Inoculum on the Reproduction of *Heterodera latipons*

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ABSTRACT

The effect of the initial inoculum on the reproduction of the Mediterranean cereal cyst nematode *Heterodera latipons* on barley was studied. Two types, cysts or second stage juveniles (J2), of initial inoculum (*Pi*) were used in this investigation. Four inoculum levels of cysts (0, 5, 10, or 20/pot filled with 500 g clay loam soil) and J2 (0, 100, 200, or 400 J2s/ pot filled with 100 g clay loam soil) were used. The results indicated that the multiplication of *H. latipons* increased as the *Pi* increased while the reproduction factor decreased. The results also showed that increasing of initial inoculum, cysts or J2, of *H. latipons* resulted in decreasing the plant height, fresh and dry weight of both shoots and roots of barley.

Keywords: Mediterranean Cereal Cyst Nematode, *Heterodera latipons*, Cysts, Pi, Pf.

INTRODUCTION

Cyst nematodes are highly pathogenic species of agronomic crops including grains, root crops and most legumes (Baldwin & Mundo-Ocampo, 1991). The damage caused by cyst nematodes varied from slight to near crop failure depending on the initial population densities and the local environmental factors (Dixon, 1969; Koenning & Schmitt, 1993; Al-Yahya *et al*., 1998; Ibrahim *et al.*, 1999). The Mediterranean cereal cyst nematode (MCCN), *H. latipons*, was found in several phytogeographical zones of Jordan (Al-Abed, 2001). In these zones, large population of MCCN were present in localities with silty clay loam, clay loam, and clay loamy soils but Al-Abed (2001) showed experimentally that the severity of this species was greater in lighter soils. The high levels of infestations in localities with different types of soil might be due to the high initial inoculum that may result from monoculturing barley for long periods. It has been reported that the increase in nematode inoculum density resulted in a higher rate of multiplication and a consequent decrease in plant growth parameters (Zancada & Althofer, 1994). In this study, the researchers investigated the effect of the initial inoculum on the reproduction of *H. latipons* and on the growth parameters of barley.

Materials and methods

The effects of different initial inoculum densities (*Pi*) of *H. latipons* on the growth of barley cv Rum and on nematode reproduction were determined under controlled conditions. Two experiments were conducted each with one type of inoculum either cyst or second-stage juveniles (J2). Nematode inoculum was prepared by extracting cysts from soil and roots of susceptible barley cultivar from naturally infested fields from Ar-Ramtha locality by floatation method (Goodey, 1963).

The first experiment was conducted by using cysts as the initial inoculum with four density levels (D0=0;

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D1=5; D2= 10; and D3= 20 cysts per pot). Five pots (10 cm diam. filled with 500 g sterilized clay loam soil) were used for each inoculum density.

The extracted cysts were added to the pots, which were moistened to field capacity and maintained at a constant temperature (10ºC) for 20 days (Mor *et al.*, 1992). After that, five seeds of the local barley cultivar "Rum" were sown in each pot. The pots were maintained in a temperature controlled growth chamber (17ºC) with a 12 h photoperiod and arranged in a completely randomized design with 5 replicates for each treatment.

A second experiment was carried out with four J2 inoculum rates (D0= 0; D1=100; D2=200; D3= 400 J2 per pot). Five pots (6 cm diam. filled with 150 g sterilized clay loam soil) were used for each inoculum density.

The J2 were obtained from cysts incubated in 6 cm diam. Petri dishes filled with tap water at 10ºC for one month, then transferred for one week at 20ºC to accelerate hatching. The hatched J2 were periodically harvested and stored at 10ºC until use. Five seeds per pot of the tested barley cv. Rum were germinated in a moist chamber. Each inoculum density was concentrated in one ml aqueous suspension and poured directly over the roots of 1-week-old barley seedlings. Five pots were used for each inoculum level. The pots were arranged in a completely randomized design in a constant temperature chamber at 17ºC and illuminated with a 12h photoperiod. The plants were harvested two months after inoculation.

The host capacity of the plants of the two experiments was assessed after they were two months old by counting the number of the white females or cysts formed. Cysts from each treatment were picked into a beaker and crushed into 20 ml of tap water. The egg suspension was then stirred on a magnetic stirrer and two 1 ml samples were removed to count the number of eggs and J2. The *Pi* (initial population) and *Pf* (final population) were expressed as the number of J2 and mean number of encysted eggs per 100 g soil. The reproduction factor (*Rf = Pf/Pi*) was determined. Plant height was determined just before harvesting. Fresh and dry weights of shoots and roots were recorded.

Data were subjected to analysis of variance (ANOVA), and means were separated by Fisher's protected LSD (Cochran & Cox, 1957). Regression analyses were also performed on data to describe the relationship of *Pi vs* shoot and root dry weight, *Pf*, and *Rf*.

Results

Results showed that the final number of cysts produced on barley cv Rum was influenced by the initial population density (Tables 1 and 2). There were significant multiplications of cysts as the initial inoculun, $Pi_{(cysts}$ increased, whereas the nematode reproductive factor (*Rf*) decreased as the $Pi_{(cysts\ or\ J2s)}$ increased. Regression analysis showed that the nematode final population ($Pf_{(cysts)}$) was positively correlated with both $Pi_{(cysts)}$ ($y = 5.385\ X + 10.68$, $r^2 = 0.95$) and $Pi_{(J2s)}$ ($y = 5.0257X + 245.56$, $r^2 = 0.93$). While, *Rf* was negatively correlated with $Pi_{(cysts)}$ ($y = -0.2143\ X + 10.14$, $r^2 = 0.98$) and $Pi_{(J2s)}$ ($y = -0.013\ X + 110.38$, $r^2 = 0.96$).

Table 1 Effects of different inoculum levels ($Pi_{(cysts)}$) on reproduction of *H. latipons* on barley cv. Rum.

Treatment* (Pi)	***Pf*** ** **(cyst)**	**Mean number of eggs per cysts**	**RF *Pf/Pi***
Control	0 d	0d	0d
5 cyst	46 c	151 a	9.24 a
10 cyst	77 b	139 ab	7.74 b

*Treatment (*Pi)*	*Pf* ** (cyst)	Mean number of eggs per cysts	RF *Pf/Pi*
20 cyst	112 a	119 b	5.94 c
LSD at *P*=0.05	13.72	29.32	1.413

* *Pi* (initial population) = number of cysts per pot (500 g soil) at the beginning of the experiment

** *Pf* (final population) = number of cysts per pot (500 g soil) at the end of the experiment.

Means in a column followed by the same letter are not significantly different (*P*< 0.05*)* according to Fisher's protected LSD.

The mean number of eggs per cyst decreased as the $Pi_{(cysts)}$ increased (Table 1) and was significantly higher when *Pi* was 5 cysts than when the *Pi* was 20 cysts per pot. While the number of eggs per cyst increased slightly with increasing $Pi_{(J2s)}$, but the differences are not significant (Table 2).

Table 2: Effects of different inoculum levels ($Pi_{(J2)}$) on reproduction of *H. latipons* on barley cv. Rum.

Treatment (*Pi*)*	*Pf* (cyst)**	*Pf* ***(eggs+J2s)	Eggs/cyst	RF (*Pf/Pi)*
Control	0 d	0 d	0 b	0 d
100 J2s	7.2c	938 c	130 a	9.4 a
200 J2s	11.0 b	1457 b	132 a	7.3 b
400 J2s	15.8 a	2105 a	132 a	5.3 c
LSD at *P*=0.05	1.56	207.9	40	1.29

* *Pi* = number of J2s per pot (150 g soil) at the beginning of the experiment.

** *Pf* (cyst) = number of cysts per pot (150 g soil) at the end of the experiment.

*** *Pf* (eggs + J2s) = number of eggs & J2s per pot (150g soil) at the end of the experiment.

Means in a column followed by the same letter are not significantly different (*P*< 0.05*)* according to Fisher's protected LSD.

The results also indicated that increasing of initial population of *H. latipons* resulted in decreasing the growth parameters of barley (Table 3 and 4). Results of the first experiment showed that the cyst nematode reduced significantly the plant height when compared to uninfested control plants. However, there were no significant differences in the height parameter of plants inoculated with different $Pi_{(cysts)}$ levels. The fresh and dry weight of both shoots and roots reduced as $Pi_{(cysts)}$ increased. Inoculating barley plants with 5 cysts /pot did not significantly reduced these growth parameters when compared to uninfested plants. On the contrary, the higher density levels (10 and 20 cysts/pot) significantly reduce these growth parameters (Table 3).

Table 3 Effect of different inoculum levels ($Pi_{(cysts)}$) of *H. latipons* on growth of barley cv. Rum.

Treatment (*Pi*)*	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
Control	45 a	5.6a	2.5 a	2.9 a	1.01 a
5 cyst	38 b	4.9 ab	2.2 ab	1.7 ab	0.74 ab
10 cyst	37 b	4.3 b	1.7 bc	1.5 b	0.42 b
20 cyst	36 b	4.0 b	1.5 c	1.6 b	0.32 b
LSD at *P*= 0.05	4.21	1.18	0.561	1.234	0.534

* *Pi* (initial population) = number of cysts per pot (500 g soil) at the beginning of the experiment.
Means in a column followed by the same letter are not significantly different ($P< 0.05$) according to Fisher's protected LSD.

The two inoculum levels, 100 and 200 J2 per pot did not significantly lower the barley plant height, but the higher density level, 400 J2s, did. The fresh weight of barley shoot was significantly reduced when plants were inoculated with the three studied inoculum levels of J2 when compared with uninfested ones. However, no a significant reduction in fresh shoot weights was observed as the *Pi* increased from 100 to 400 J2 per pot. The same trend was observed on dry weight of roots. The two higher levels of *Pi* (200 and 400 J2 per pot) significantly reduced both dry shoot and fresh root weights (Table 4)

Table 4 Effects of different inoculum levels ($Pi_{(J2s)}$) of *H. latipons* on growth of barley cv. Rum.

Treatment (*Pi*)*	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
Control	39 a	1.9 a	1.1 a	0.8 a	0.18 a
100 J2s	38 ab	1.4 b	0.8 ab	0.5 ab	0.12 b
200 J2s	37 ab	1.3 b	0.6 b	0.4 bc	0.10 b
400 J2s	34 b	1.2 b	0.7 b	0.1 c	0.09 b
LSD at *P*= 0.05	4.62	0.23	0.32	0.33	0.043

* *Pi* = number J2s per pot (150 g soil) at the beginning of the experiment
\Means in a column followed by the same letter are not significantly different ($P< 0.05$) according to Fisher's protected LSD.

Discussion

The *Pf* of *H. latipons* was positively correlated with the increased *Pi* (cysts or J2). Similar findings on *H. avenae*, a close relative of *H. latipons*, were also reported (Zancada & Althofer, 1994 Al-Yahya *et al*., 1998; Ibrahim *et al*., 1999). By contrast, the reproductive factor was negatively correlated with *Pi*. This statistic was in agreement with those reported by several investigators (Lamondia & Brodie, 1986; Rioval & Sarr, 1987; Greco & Brandonisio, 1987; Al-Hazimi, *et al*., 1999; Ibrahim *et al*., 1999)**.** The negative correlation between *Rf* and *Pi* could be due to the competition for the feeding sites, and the greater damage of infected roots with increasing (*Pi*). Thus, there will be a

reduction of the suitable areas of the roots for nematode infection, establishment, and reproduction (Lamondia & Brodie, 1986).

In this study, both inoculum types (cysts or J2) gave similar results. The use of *Pi* as cysts was done to resemble the natural infestation. However, using J2 as a source of inoculum might be better for obtaining uniform conditions. O'Brien and Fisher (1979) reported that in order to study the effects of different initial inoculum densities of *H. latipons* under uniform conditions of inoculation, young active juveniles of similar age should be used as the inoculum.

The plant parameter measured here was adversely affected by the *Pi.* The relationship between the initial population density (*Pi*) of nematodes and growth of the host plant is very important in determining the economic impact of the nematode on this crop. Several reports showed that increasing nematode densities resulted in an increase in the incidence and the severity of plant diseases. It has been estimated that every increase of 10 egg/g soil of *H. avenae* caused yield losses of 188 kg/ha of barley in the UK (Dixon, 1969). Another study showed that in temperate semi-arid regions of Australia, *H. avenae* decreased the yield of wheat and barley by 20% at *Pi* of 2 eggs and juveniles per g soil, and 40% at 16 eggs and juveniles per g soil (Meagher & Brown, 1974). Moreover, Zancada and Althofer (1994) found that *H. avenae* applied at two inoculum levels (15 and 25 cysts per 7 l pot) delayed plant development. This delay resulted in later ripening, thus making plants more susceptible to periods of water stress in areas with a Mediterranean climate. It is important to conduct experiment in the field to determine the yield loss and then relate *Pi* and *Pf* to yield loss.

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تأثير العدوى الأولية على تكاثر النيماتودا الحوصلية *Heteroderalatipons*

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ملخـص

تم دراسة تأثير العدوى الأولية لنيماتودا المحاصيل الحقلية الحوصلية على تكاثر النيماتودا الحوصلية على الشعير. تم استخدام الحوصلات أو الطور اليرقي الثاني (J2) كعدوى أولية حيث تم اختبار أربعة مستويات من الحوصلات (0، 5، 10، 20 /قوار معبأ بحوالي 500غم تربة) وأربعة مستويات من الطور اليرقي الثاني (0، 100، 200، 400 يرقة /قوار معبأ بحوالي 100 غم تربة). أظهرت النتائج أن تكاثر نيماتودا المحاصيل الحقلية الحوصلية ازداد مع ازدياد العدوى الأولية بينما انخفض معامل التكاثر، كذلك أظهرت النتائج أن ازدياد العدوى الأولية سواء كانت حوصلات أو يرقات كانت لها تأثيرات سلبية على نمو الشعير حيث أدت إلى تقليل الوزن الجاف و الطري لكل من المجموع الخضري و الجذري.

الكلمات الدالة: نيماتودا المحاصيل الحقلية المتوسطية، *Heteroderalatipons*، حويصلات، Pf, Pi، العدوى الأولية.

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