

## PARASITIZATION OF MAIZE BY *HETERODERA AVENAE* AND *H. FILIPJEVI*

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**Summary.** Fewer second stage juveniles of *Heterodera avenae* and *H. filipjevi* penetrate the roots of winter maize than penetrate wheat roots. The majority of the penetrated juveniles develop to adulthood in primary roots but females remain small, trapped inside the roots, and are unable to reproduce. Developing males mostly remain inside the roots in February-sown plants but emerge into the soil in December-sown plants. The adult stage of both nematode species is reached in about 5 weeks in February-sown and in 8-9 weeks in December-sown plants of wheat as well as maize. Some of the second stage juveniles entering the stelar region in February-sown plants are surrounded by necrotic root tissues and fail to develop beyond this stage. Maize roots penetrated by *H. avenae* become hard and stubby; *H. filipjevi* infected roots become swollen, develop lateral branches but continue to grow. Winter maize can be exploited as a trap crop for the management of *H. avenae* and *H. filipjevi*.

Cereal cyst nematodes, the *Heterodera avenae* complex, are important nematode pests of wheat, oats and barley in many countries of the world. Populations from Germany, England, Sweden and India that were previously known as race 3, pathotype 3, pathotype B or the Gotland strain of *H. avenae* Woll. are now considered to belong to *H. filipjevi* Madzhidov (Subbotin *et al.*, 1996; Sturhan and Rumpfenhorst, 1996; Bekal *et al.*, 1997; Rivoal *et al.*, 2003; Bishnoi and Bajaj, 2004). Several graminaceous plants have been reported as hosts for *H. avenae*; however, there are divergent reports regarding the host status of maize to this nematode. Rivoal (1975) found that all pathotypes prevalent in France attack maize roots but fail to reproduce. Saefkow and Lücke (1979) and Maas and Brinkman (1977) recorded normal development and reproduction on maize roots of *H. avenae* populations from Germany (pathotype E) and Holland, respectively. However, Behringer (1978) observed that second stage juveniles ( $J_2$ ) of *H. avenae* penetrate but do not mature in maize roots in Germany. Johnson and Fushtey (1966) made detailed studies on the development of *H. avenae* on maize and on a susceptible variety of oat, and concluded that females developing on maize remain small-sized and hence fail to protrude from the roots for reproduction.

On the northern plains of India, maize is cultivated mainly as a summer crop from June-July to October. This crop is also grown in the winter season (November to April) and as a fodder crop from February-March to May-June. Second stage juveniles of indigenous populations of *H. avenae* and *H. filipjevi* remain in diapause during summer (April to October) and start to hatch

when the temperature declines in November (Fig. 1), coinciding with the season in which wheat, barley and winter maize crops are sown. There is no unanimity regarding the host status of maize to Indian populations of the former nematode species. Maize has been rated as a non-host for *H. avenae* with no penetration by second stage juveniles (Bhatti *et al.*, 1977), a poor host (Gill and Swarup, 1971) or, in contrast, a good host (Swarup *et al.*, 1964; Yadav and Verma, 1971). Preliminary studies conducted by Kanwar *et al.* (2002) with Indian populations of *H. avenae* and *H. filipjevi* indicated that  $J_2$  of both nematode species readily infect the roots of maize. Further investigations were, therefore, planned to compare the development of these nematode species in maize and in wheat.

### MATERIALS AND METHODS

The initial studies were conducted during February-April, 2004. Pre-germinated seeds of maize variety HPQM 1 and wheat variety WH 147 were sown separately, in earthenware pots containing one kg steam-sterilized soil. Each pot, having one plant, was inoculated with 1000 hatched  $J_2$  of *H. avenae* (Udaipur population) or 200  $J_2$  of *H. filipjevi* (Ludhiana population). The experiment was repeated during December, 2004-March, 2005 but with an inoculum level of 500  $J_2$  per pot for both species. The juveniles were obtained by incubating the cysts in distilled water at 10 °C for 25 days and collecting  $J_2$  every third day. Observations on nematode penetration and development were recorded periodically, as given in Tables I and II. Nematode penetration and development were studied by staining the roots in 1% acid-fuchsin lactophenol. The soil of each pot

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was processed through a 60-mesh sieve placed over a 300-mesh sieve by Cobb's decanting and sieving method. White females and cysts remaining on the 60-mesh were collected and counted. The catch of the 300-mesh sieve was further processed by Baermann's funnel technique for the recovery of males.

Average maximum and minimum air temperature data, recorded by Agricultural Meteorology Department, CCS Haryana Agricultural University, Hisar, are reported to highlight the differences between the two experimental periods.

## RESULTS

Second stage juveniles of *H. avenae* and *H. filipjevi* readily penetrated the maize and wheat roots in both experiments. However, more  $J_2$  of both species had penetrated wheat roots than maize roots 15 days after inoculation (DAI) (Tables I, II), and no juveniles were observed leaving the maize roots as reported for *H. avenae* in resistant varieties of oats or wheat (Cook and Mizen, 1991; Kanwar *et al.*, 2005).

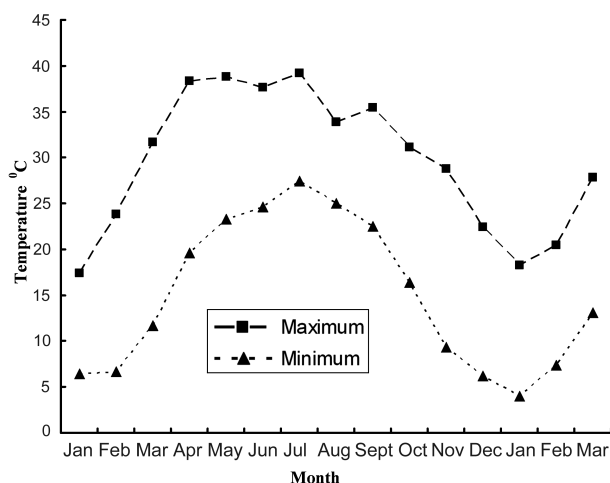
*February-sown plants.* In wheat, more than 90% of the nematodes in the roots were in the  $J_3/J_4$  stages by 30 DAI. Adult males and white females of *H. avenae* and *H. filipjevi* were recovered from roots and soil, 35 and 40 DAI, respectively. Adult males or females were recovered from soil only 45 DAI. Roots cortex had sloughed off by this time and as such roots contained no nematode.

In maize roots, only about 10% of the juveniles of *H. avenae* were in  $J_3$  and  $J_4$  stages at 30 DAI. Most of the  $J_2$  had penetrated the first emerging primary roots, which then became stubby. Penetration rarely occurred in secondary and tertiary roots. No lateral branching (as occurs in wheat roots) took place at penetration sites. Only one male was recovered from the soil, 35 DAI, though

roots harboured all developmental stages of nematode. At 45, 60 and 70 DAI, no nematode was recovered from the soil while primary roots contained males, females and even some  $J_2$ . The nematodes inside the secondary or tertiary roots had either disintegrated or were still at  $J_2$  or  $J_3$  stages. The females teased out from roots of maize were smaller [628 (614-633) × 411 (376-435)  $\mu\text{m}$ ] than those from wheat roots [779 (570-874) × 576 (280-684)  $\mu\text{m}$ ]. The length of the neck was inversely related to the size of females. The majority of  $J_2$  penetrating maize roots developed normally to adult females or males but quite a few of those that entered the stelar region were surrounded by necrotic tissues and failed to develop beyond  $J_2$ , and could still be seen 60 and 70 DAI.

The development of *H. filipjevi* was similar to that of *H. avenae*, except that more males (40) than females (4) were found in roots of maize 40 DAI and infected roots continued to grow and did not become stubby. Usually, infected parts of roots became swollen and developed lateral branches. Females of *H. filipjevi* that developed on maize were smaller, 579 (574-594) × 307 (277-356)  $\mu\text{m}$ , than those that developed on wheat, 616 (608-646) × 448 (418-456)  $\mu\text{m}$ .

*December-sown plants.* The development of both *H. avenae* and *H. filipjevi* on wheat and maize roots was similar to, but slower than, in February-sown plants. Adult females were present in roots of both plant species by 60 DAI as compared to 35/40 DAI in February-sown plants (Table II). The other notable difference in maize was the ability of a good number of males to leave the roots and emerge into the soil. This variation in nematode behaviour between February- and December-sown plants might be due to differences in prevailing temperatures during the two seasons (Fig. 1). Some males remained inside the roots even at 80 DAI, and four or five males were occasionally observed in the vicinity of a single female.



**Fig. 1.** Average monthly minimum and maximum air temperature during January, 2004-March, 2005 (Source: Agricultural Meteorology Department, CCS Haryana Agricultural University, Hisar).

## DISCUSSION

A large number of cyst nematode females developed inside the primary roots of February- and December-sown maize plants but nearly all of them remained trapped inside the roots and failed to protrude, as also observed by Johnson and Fushtey (1966). Bajaj *et al.* (1996) also recorded similar observations while studying the development of *H. avenae* on the resistant barley variety C164.

A few females of *H. avenae* and *H. filipjevi* collected from inside the maize roots contained eggs. Since reproduction in both these species is by amphimixis, such females might have been inseminated by males that remained inside the roots. The inability of males and females to emerge or protrude from roots and their subsequent absence from the soil also explains the results obtained by earlier workers, who reported that *H. avenae* entering into the roots of maize do not mature

**Table I.** Developmental stages of *Heterodera avenae* and *H. filipjevi* in maize var. HQPM 1 and wheat var. WH 147 during February to April, 2004.

Days after inoculation	<i>Heterodera avenae</i>				<i>Heterodera filipjevi</i>			
	Maize		Wheat		Maize		Wheat	
	Root system	kg soil	Root system	kg soil	Root system	kg soil	Root system	kg soil
15	101 J <sub>2</sub> , 1 J <sub>3</sub>	0	125 J <sub>2</sub> , 1 J <sub>3</sub>	0	19 J <sub>2</sub>	0	24 J <sub>2</sub> , 1 J <sub>3</sub>	0
30	98 J <sub>2</sub> , 7 J <sub>3</sub> , 3 J <sub>4</sub> ♀	0	10 J <sub>2</sub> , 77 J <sub>3</sub> , 19 J <sub>4</sub> ♀, 15 J <sub>4</sub> ♂	0	19 J <sub>2</sub> , 1 J <sub>3</sub>	0	2 J <sub>2</sub> , 20 J <sub>3</sub> , 4 J <sub>4</sub> ♀	1 ♂
35	36 J <sub>2</sub> , 9 J <sub>3</sub> , 20 J <sub>4</sub> ♀, 11 J <sub>4</sub> ♂, 5 ♀, 3 ♂	1 ♂	14 J <sub>2</sub> , 10 J <sub>3</sub> , 27 J <sub>4</sub> ♀, 18 J <sub>4</sub> ♂, 12 ♀, 3 ♂	1 ♀, 13 ♂	-	-	-	-
40	4 J <sub>2</sub> , 4 J <sub>3</sub> , 6 J <sub>4</sub> ♀, 3 J <sub>4</sub> ♂, 10 ♀, 5 ♂	0	17 ♀	4 ♀, 1 ♂	5 J <sub>2</sub> , 7 J <sub>4</sub> ♀, 4 ♀, 40 ♂	0	15 ♀, 2 ♂	2 ♀, 5 ♂
45	21 J <sub>2</sub> , 4 J <sub>3</sub> , 5 J <sub>4</sub> ♀, 6 J <sub>4</sub> ♂, 19 ♀, 17 ♂	0	Cortex sloughed off	23 ♀, 7 ♂	-	-	-	-
50	8 J <sub>2</sub> , 2 J <sub>3</sub> , 4 J <sub>4</sub> ♀, 3 J <sub>4</sub> ♂, 18 ♀, 14 ♂	1 ♂	Cortex sloughed off	17 ♀, 9 ♂	10 J <sub>2</sub> , 12 ♀, 23 ♂	1 ♀	Cortex sloughed off	17 ♀
60	2 J <sub>2</sub> , 1 J <sub>3</sub> , 15 ♀, 18 ♂	0	Cortex sloughed off	20 cysts	2 J <sub>2</sub> , 10 ♀, 15 ♂	0	Cortex sloughed off	16 cyst
70	3 J <sub>2</sub> , 13 ♀, 12 ♂	0	Cortex sloughed off	24 cysts	8 ♀, 10 ♂	0	Cortex sloughed off	15 cysts

Abbreviations: J<sub>2</sub> = Second stage juvenile, J<sub>3</sub> = Third stage juvenile, J<sub>4</sub> ♀ = Fourth stage female juvenile, J<sub>4</sub> ♂ = Fourth stage male juvenile, ♀ = Adult female, ♂ = Adult male.

**Table II.** Development of stages of *H. avenae* and *H. filipjevi* in maize var. HQPM 1 and wheat var. WH 147 during December, 2004 to March, 2005.

Days after inoculation	<i>Heterodera avenae</i>				<i>Heterodera filipjevi</i>			
	Maize		Wheat		Maize		Wheat	
	Root system	kg soil	Root system	kg soil	Root system	kg soil	Root system	kg soil
15	45 J <sub>2</sub>	0	152 J <sub>2</sub>	0	40 J <sub>2</sub>	0	226 J <sub>2</sub>	0
25	65 J <sub>2</sub>	0	167 J <sub>3</sub>	0	82 J <sub>2</sub> , 7 J <sub>3</sub>	0	21 J <sub>2</sub> , 103 J <sub>3</sub>	0
35	18 J <sub>2</sub> , 18 J <sub>3</sub> , 4 J <sub>4</sub> ♀, 2 J <sub>4</sub> ♂	0	114 J <sub>3</sub> , 14 J <sub>4</sub> ♀, 15 J <sub>4</sub> ♂	0	15 J <sub>2</sub> , 30 J <sub>3</sub>	0	212 J <sub>3</sub> , 11 J <sub>4</sub> ♀, 13 J <sub>4</sub> ♂	0
45	37 J <sub>2</sub> , 2 J <sub>3</sub> , 3 J <sub>4</sub> ♀, 3 J <sub>4</sub> ♂	0	31 J <sub>3</sub> , 60 J <sub>4</sub> ♀, 69 J <sub>4</sub> ♂	0	48 J <sub>2</sub> , 7 J <sub>3</sub> , 8 J <sub>4</sub> ♀, 5 J <sub>4</sub> ♂	0	65 J <sub>3</sub> , 34 J <sub>4</sub> ♀, 21 J <sub>4</sub> ♂	0
60	3 J <sub>3</sub> , 4 J <sub>4</sub> ♀, 7 J <sub>4</sub> ♂, 25 ♀, 5 ♂	0	12 J <sub>3</sub> , 52 J <sub>4</sub> ♀, 28 J <sub>4</sub> ♂, 8 ♀	2 ♀, 28 ♂	2 J <sub>2</sub> , 9 J <sub>3</sub> , 10 J <sub>4</sub> ♀, 10 J <sub>4</sub> ♂, 1 ♀	0	2 J <sub>3</sub> , 30 J <sub>4</sub> ♀, 40 J <sub>4</sub> ♂, 8 ♀	4 ♀, 3 ♂
70	3 J <sub>3</sub> , 6 J <sub>4</sub> ♀, 7 J <sub>4</sub> ♂, 19 ♀, 14 ♂	2 ♂	22 ♀	7 ♀, 44 ♂	2 J <sub>3</sub> , 3 J <sub>4</sub> ♀, 36 ♀, 13 ♂	1 ♂	3 J <sub>4</sub> ♀, 10 ♀, 5 ♂	17 ♀, 33 ♂
80	1 J <sub>3</sub> , 4 J <sub>4</sub> ♀, 2 J <sub>4</sub> ♂, 19 ♀, 10 ♂	13 ♂	Cortex sloughed off	68 ♀	1 J <sub>4</sub> ♀, 12 ♀, 2 ♂	18 ♂	Cortex sloughed off	24 ♀, 5 ♂

Abbreviations: J<sub>2</sub> = Second stage juvenile, J<sub>3</sub> = Third stage juvenile, J<sub>4</sub> ♀ = Fourth stage female juvenile, J<sub>4</sub> ♂ = Fourth stage male juvenile, ♀ = Adult female, ♂ = Adult male.

(Behringer, 1978) or that only males develop in maize roots (Hirling, 1974).

Dalal *et al.* (1988) and Kanwar *et al.* (2002) reported occasional production of small cysts of *H. avenae* containing a few eggs by some Indian populations on winter maize varieties. Production of small-sized cysts with few eggs by cereal cyst nematodes on resistant hosts is a common phenomenon (Cook and Mizen, 1991; Bajaj *et al.*, 1996; Kanwar *et al.*, 2003). However, Saefkow and Lücke (1979), working with pathotype E (= Ha 61) in Germany, found that this nematode developed normally on maize and cysts containing eggs with J<sub>2</sub> were formed 12-13 weeks after initial root invasion. This difference could be attributed to differences in the pathotype virulence and/or susceptibility of the maize variety (Lücke and Saefkow, 1978). Swarup *et al.* (1964) and Yadav and Verma (1971) reported reproduction of *H. avenae* on maize in India. However, Bhatti *et al.* (1977) did not observe root penetration by juveniles or reproduction of *H. avenae* (Mahendergarh, Haryana population) on maize grown from June to October. Summer maize is unlikely to be attacked by *H. avenae* or *H. filipjevi* as they remain in diapause during this season under natural conditions on the northern plains of India. J<sub>2</sub> of *H. avenae* (obtained by incubating cysts at 10 °C) penetrated maize and wheat roots during August, 2004 but failed to develop beyond J<sub>2</sub> (with no swelling) even by 20 DAI (unpublished data). The present results agree with previous observations by Maas and Brinkman (1977), who also reported that the time of sowing of maize influences the reproduction of *H. avenae* and attributed this to the activity of the juveniles. Bajaj *et al.* (1986) also observed a pronounced effect of temperature on the development of *Heterodera zaeae* on wheat and maize. It is worth mentioning here that *H. zaeae* was described from India by Koshy *et al.* (1970) and the concomitant occurrence of *H. zaeae* and *H. avenae* is common in India (Koshy and Swarup, 1971; Bajaj and Walia, 1985). It is quite probable that Swarup *et al.* (1964) and Yadav and Verma (1971) worked with mixed populations of both species, when reporting reproduction of *H. avenae* on maize.

The present studies reveal that both *H. avenae* and *H. filipjevi* can readily infect and develop on winter maize, but that they fail to reproduce as females remain trapped inside the roots. Consequently, winter maize can be exploited as a trap crop for the control of *H. avenae* and *H. filipjevi* on wheat and barley in India. Reduction of infestation in wheat fields by growing maize has also been reported by Rivoal (1973), Bachthaler and Hien (1976) and Behringer (1978).

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