# SEASONAL VARIATION OF FIELD POPULATIONS OF HETERODERA FILIPJEVI, PRATYLENCHUS THORNEI AND P. NEGLECTUS ON WINTER WHEAT IN TURKEY

## E. Sahin<sup>1\*</sup>, J.M. Nicol<sup>2</sup>, A. Yorgancilar<sup>1</sup>, I.H. Elekcioglu<sup>3</sup>, A. Tulek<sup>4</sup>, A.F. Yildirim<sup>5</sup> and N. Bolat<sup>1</sup>

<sup>1</sup> Anatolian Agricultural Research Institute, Karabayir Baglari, P. O. Box 17, 26002 Eskisehir, Turkey
<sup>2</sup> CIMMYT (International Maize and Wheat Improvement Centre), P.O. Box 39, Emek 06511 Ankara, Turkey
<sup>3</sup> Cukurova University, Faculty of Agriculture, Department of Plant Protection, Balcali Adana, Turkey
<sup>4</sup> Trakya Agricultural Research Institute, P.O. Box 16, 22100 Edirne, Turkey
<sup>5</sup> Plant Protection Central Research Institute, Bagdat Str. No. 250, Yenimahalle 06170 Ankara, Turkey

**Summary.** The development of cereal cyst nematode *Heterodera filipjevi* and root lesion nematodes *Pratylenchus thornei* and *P. neglectus* was investigated under rainfed cereal conditions over three growing seasons (2002-2005) on the winter wheat cultivar Bezostaya. Juvenile emergence of *H. filipjevi* was recorded during the winter period from November to March. The hatching process was correlated with the lowest temperatures. Mature white females were found on roots at the beginning of May and mature cysts appeared later on. The total number of cysts and eggs in the soil had only one peak at the end of each growing season, suggesting that *H. filipjevi* was monocyclic. Multiplication rates were inversely correlated with initial nematode densities with ceiling levels of between 15 and 20 eggs per g of dry soil. Population densities of *P. thornei* and *P. neglectus* were low from November to March/April during the cold snow period, increased gradually to June/July and then rapidly decreased over the summer period. Numbers of nematodes were positively correlated with temperature and the multiplication rate was 0.42-3.8 for *P. thornei* and 0.91-2.26 for *P. neglectus*.

Key words: Cereal cyst nematode, hatching cycles, population dynamics, root-lesion nematodes.

The sedentary Cereal Cyst Nematodes (CCN - Heterodera spp.) and the migratory Root Lesion Nematodes (RLN - Pratylenchus spp.) are globally important plant parasitic nematodes on small grain cereals (Nicol et al., 2003). The Heterodera avenae Woll. group consists of twelve valid species. The most economically important ones on cereals are Heterodera avenae Wollenweber, H. latipons Franklin and H. filipjevi Madzhidov (Abidou et al., 2005; McDonald and Nicol, 2005). Recent studies in Turkey have estimated that up to 50% yield loss is caused by H. filipjevi to commonly cultivated winter wheat varieties in the Central Anatolian Plateau (CAP) (Nicol et al., 2004). Eight species of RLN are known to be parasitic on small grain cereals. Of these, Pratylenchus thornei Sher et Allen, P. neglectus Rensch and P. penetrans Cobb are the most economically important (McDonald and Nicol, 2005). Damage thresholds for H. avenae have been stated to be 0.2, 1 and 5 eggs + juveniles/g of soil for oats, wheat and barley, respectively, in temperate regions (Andersen, 1961; Franklin, 1951). Soil populations of P. thornei of more than 2.5 nematodes/g soil are considered to be damaging population densities in Australia (Vanstone et al., 1998).

An appropriate integrated control strategy is recommended to keep nematode populations below economic damage thresholds. It is well recognized that crop rotation, host resistance and tolerance are the only economically and environmentally sustainable methods of controlling these nematodes under field conditions. Development of an effective integrated control strategy in a given area requires a good understanding of both the biology and population dynamics of the causal nematodes (Brown, 1987).

The biology of the obligate parasite *H. avenae* coincides with the cereal growing period and produces one generation per year (Rivoal and Cook, 1993), but there is no published information on the biology of *H. filipje-vi* under rainfed agro-ecological conditions. On the other hand, RLN have multiple generations per year, increasing the nematode population densities throughout the growing season (Nicol, 1996).

Therefore, for the first time in Turkey, the population dynamics of *H. filipjevi*, *P. thornei* and *P. neglectus* on wheat were monitored for three consecutive growing seasons (2002-2005) in a naturally infested experimental field in CAP.

## MATERIALS AND METHODS

*Research site and trial.* The population dynamics of *H. filipjevi, P. thornei* and *P. neglectus* were investigated in a naturally infested experimental field located at Haymana near Ankara (latitude 39 24' 13" and longitude 32 37' 14"). The climate in this region is continental, wet and cold in winter, hot and dry in summer. Winter wheat is planted in late September/early October and harvested in July/August.

<sup>\*</sup> Corresponding author e-mail: e.sahin@cgiar.org

Prior to the study, *H. filipjevi* was identified as the only CCN group species present in the research area, based on morphological and molecular PCR/RFLP identification of the cysts (Hooper, 1986; Yorgancilar *et al.*, 2005). Identification of *P. thornei* and *P. neglectus* was made morphologically (Townshend and Anderson, 1976; Fortuner, 1977).

The trial consisted of five replicated field plots (1.2 m wide – six rows  $\times$  7 m) cultivated with susceptible winter wheat cultivar Bezostaya-1. While the positions of the plots varied slightly from year to year, they were always located within 50 m of their position in the previous year. Eight, fifteen and thirteen soil samples were collected at 2-week intervals in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> years

of the experiment, respectively, over the wheat growing season. Composite soil samples of 16 cores were collected using a 2 cm diameter corer to a depth of 20 cm in each plot in a random manner between plant rows on each occasion. The number of cysts and second stage juveniles of cereal cyst nematode and the number of root lesion nematodes in the soil samples were assessed. Six plant root samples per plot were also examined for the presence of endoparasitic nematode stages at each sampling time. Multiplication rates of the three nematodes were calculated by the ratio of the final population density in July to the initial soil population density in November. Initial population densities in March were used only in 2004 for the calculation of multiplication rates



**Fig. 1.** A: Monthly mean temperature (°C) at 20 cm in the soil (line  $\Box$ ) and monthly total rainfall (mm) (bars) from November 2002 to July 2005. B: Numbers and significant highest densities (\*) of *Heterodera filipjevi*. C: Numbers and significant highest densities of *Pratylenchus thornei* (\*) and *P. neglectus* (+).

for *P. thornei* and *P. neglectus*, due to their undetectable level in the soil in November.

Assessment of Pratylenchus spp. and H. filipjevi in the soil. Motile stages of both Pratylenchus species and second-stage juveniles of *H. filipjevi* in the soil were extracted using the Whitehead tray technique (Whitehead and Hemming, 1965). The final counts were expressed as numbers of nematodes per 200 g of dry soil for the different species of RLN and per g of dry soil for *H. filipjevi*.

Assessment of H. filipjevi cysts and encysted eggs in the soil. Cysts of H. filipjevi were extracted from 250 g dry soil samples using the Fenwick can technique (Fenwick, 1940), modified according to Stirling *et al.* (1999). The numbers of full and empty cysts were counted for each sample. Average egg content per cyst was estimated by taking 50 individual newly formed full cysts at random at the end of each growing season. Each cyst was individually broken and the egg and juvenile content counted. Densities of eggs per g of dry soil at each sampling time were determined by multiplying the number of full cysts with the average egg content per cyst.

Assessment of CCN and RLN in the roots. Roots of six plants from each plot, at each sampling time, were examined visually for further developmental stages (white females) of *H. filipjevi* and then processed for extraction of mobile nematodes with the mistifier method (Stirling *et al.*, 1999). Roots were washed under running tap water and divided at random into duplicate subsamples with three plants in each. Roots of each subsample were cut into 1 cm long pieces and placed in the extraction chamber. Numbers of *Pratylenchus* specimens were counted.

*Analysis of data*. Numbers of *P. thornei* and *P. neglectus* per 200 g of dry soil, total numbers of eggs and numbers of hatched juveniles per g of dry soil for *H. filipjevi* throughout the experiment were analyzed using ANOVA (SAS Institute, 1985, Cary, NC, USA). Significant differences were calculated at P < 0.05.

#### RESULTS

## Temperature and precipitation

The average temperature at 20 cm depth in the soil was 6 °C from November to March/April and rose to an average of 21 °C from May to July. Precipitation was snow from November/December to March and rain from April to July. Average monthly precipitation was 21.8 mm from November to March and 17.3 mm from April to July during the 2002-2005 periods (Turkish State Meteorological Service, 2006, Ankara, Turkey, Fig. 1A).

#### Heterodera filipjevi

*Hatching cycle.* Motile second-stage juveniles were found in the soil during the coldest period, from November to March, in all three experimental years. Significant juvenile emergence was recorded in November 2002 (1.5 juveniles/g soil), in November 2003 (2.3 juveniles/g soil) and in March 2004 (2.8 juveniles/g soil). The emergence of *H. filipjevi* juveniles was greatest (more than 5 juveniles/g of soil) during the 2004-2005 growing season, beginning in November 2004, peaking in February 2005 (P< 0.05) and decreasing between March and April. In addition, minor hatching occurred during April and June 2005, which was not observed in 2003 and 2004 (Fig. 1B).

During the main hatching period, mean monthly soil temperatures were the lowest, ranging from 9 °C in November 2002 to 2 or 3 °C in January 2004 and February 2005, respectively. Juvenile emergence does not coincide with the highest precipitations (Fig. 1A). In contrast, the strong relationship (R = 0.61) between juvenile emergence and lower temperatures demonstrated that hatching of *H. filipjevi* occurred essentially during the cold phase (autumn-winter) of the wheat growing period (Fig. 2).



**Fig. 2.** Relationship between the monthly mean temperature at 20 cm depth in the soil and hatching of *H. filipjevi* over the three growing seasons (November 2002-July 2005).

*Development cycle.* Juveniles were found in roots until April. White females were observed on the roots at the beginning of May in each growing season (results not shown). The greatest numbers of cysts (38-49 in 250 g of dry soil) and encysted eggs (66-85 per g of soil) were observed in June-July of the growing seasons 2003 and 2004. These data show that the population of *H. filipjevi* (cysts and eggs) presented only one peak per growing season (Fig. 3).

*Multiplication rate.* The ratio of the numbers of eggs counted in July (Pf) to the number of eggs counted in the previous November (Pi) allowed estimation of the multiplication rates of *H. filipjevi*. Results from the three growing seasons (2002-2005) gave a wide range of initial soil



**Fig. 3.** Densities of *H. filipjevi* (eggs: □ and cysts: ◆) over the three growing seasons (November 2002-July 2005). Significant highest values for eggs (\*).

population densities (2-33 eggs) per gram of soil. The multiplication of *H. filipjevi* was inversely related to the initial population density and ratios ranged from 20 to 30 at initial densities lower than five eggs per gram of soil to one at more than fifteen eggs per g of soil (Fig. 4).

#### Pratylenchus thornei and P. neglectus

Development cycle. Population densities of *P. thornei* and *P. neglectus* were small at the beginning of the experiment in November 2002, ranging from 20 to 70 nematodes per 200 g of soil (Fig. 1C). Thereafter they increased gradually to reach the greatest numbers in 2003 during June and July, when up to 100 nematodes per 200 g of dry soil were found, but numbers remained low during the following (2004) growing season, with no more than 30 nematodes per 200 g dry soil for *P. neglectus*. Nevertheless, population densities, particularly those of



**Fig. 4.** Relations between the multiplication rates of *Heterodera filipjevi* and initial densities (Pi) on the wheat cultivar Bezostaya during the three growing seasons (November 2002 – July 2005).

*P. neglectus*, increased significantly in 2005 to more than 100 nematodes per 200 g of soil in the June sampling.

Densities of both *P. thornei* and *P. neglectus* were positively correlated with soil temperatures (Fig. 5); however, the relationship was stronger for *P. neglectus* (R = 0.59) than for *P. thornei* (R = 0.43). The numbers of nematodes in the soil were not significantly correlated with precipitation. The numbers of *Pratylenchus* extracted from the root tissues were variable and, therefore, are not presented. All developmental stages of the nematodes were found in the roots throughout the three growing seasons and the greatest numbers of nematodes were extracted from roots in May and June in all three years.

*Multiplication rates.* The multiplication rates were between  $0.4 \times$  and  $3.8 \times$  for *P. thornei* and  $0.9-2.3 \times$  for *P. neglectus* in the three years of the experiment. There



**Fig. 5.** Relationship between the monthly mean temperature at 20 cm depth in the soil and densities of *Pratylenchus neglectus* (\_\_\_, R = 0.59) and *Pratylenchus thornei* (..., R = 0.43) over the three growing seasons (November 2002-July 2005).

was a logarithmic relationship between initial population densities and multiplication rates for both species for the three years (R = 0.30 for *P. thornei* and R = 0.32for *P. neglectus*) (results not shown).

## DISCUSSION

*Heterodera filipjevi*. The major factors that influence the hatching of cereal cyst nematode include soil temperature, rainfall and, with a relatively small effect, root exudates of host plants (Clarke and Perry, 1977). In most reports the major hatch of CCN occurs during the growing season, once seeds have germinated and moisture is available and usually in association with lower temperatures. Nevertheless, contrasting peaks of hatching were observed between northern and southern populations of *H. avenae* in France, with a winter activity for the Mediterranean ecotype and a spring activity for the northern ecotype located in more or less temperate regions. For both ecotypes hatching occurred mostly at temperatures below 10 °C (Rivoal, 1982, 1986). Hatching of Australian populations of *H. avenae* was observed after June (in late autumn and winter) for the southern hemisphere, after the temperatures dropped to 10 °C (Meagher, 1970; Banyer and Fisher, 1971). Similarly, in Syria, the major hatch of H. latipons was at 10 °C in January/February (Scholz and Sikora, 2004).

In Turkey, juveniles of *H. filipjevi* emerged from the cysts one month after planting the crop, also when temperature decreased below 10 °C in November. Hatching continued during winter, when temperatures were at their lowest until the beginning of spring. Rising temperatures (above 15 °C) in March were regularly followed by an arrest of hatching in all three years of the experiment. Clarke and Perry (1977) pointed out that soil moisture contributes to nematode activity in the soil and Meagher (1970) observed that low rainfall delayed the emergence of juveniles. In our study, emergence peaks of juveniles were not strictly correlated with the highest rainfall period, suggesting that during the snow period natural soil moisture was sufficient to allow hatching to proceed.

Juveniles were extracted from the wheat roots in April but were small in number later on. By May, white females appeared, which later turned to brown cysts and fell into the soil in summer when the wheat roots died. Only one peak of total numbers of cysts and eggs in the soil was observed at the end (in June/July) of each growing of the wheat in this three year study. These results demonstrated, as previously reported (Rivoal, 1986; Scholz and Sikora, 2004) for the closely related species *H. avenae* and *H. latipons*, that *H. filipjevi* is also a monocyclic nematode in Turkey.

As previously demonstrated for other cereal cyst nematodes (Rivoal and Saar, 1987), multiplication rates of *H. filipjevi* were inversely correlated with initial densities. The highest rates were observed when the initial densities were the lowest, whilst when initial population densities of the nematodes were large the population at the end of the crop cycle remained unchanged. The ceiling level of the nematode population density was relatively low at around 15-20 eggs per g of dry soil. This decrease of the multiplication rate of the nematode with increase of initial population density could be due to competition between juveniles for food and space in wheat roots (Seinhorst, 1966).

Pratylenchus thornei and P. neglectus. The optimum temperature for embryogenesis of both P. thornei and P. neglectus is stated to be 25 °C (Vanstone and Nicol, 1993). It is also reported that *P. thornei* completes its life cycle at 27 °C in 40-45 days (Nicol, 1996) and the adults of P. neglectus develop in 35-40 days under natural conditions depending on the plant type, temperature, moisture and other environmental conditions (Taylor and Vanstone, 1996). Based on this information and with a winter wheat environment with inherent lower soil temperatures, it is expected that two or three cycles of RLN could be completed during the growing season on our experimental site. Elekcioglu and Gözel (1997) found that the numbers of *P. thornei* in the soil was low until March and then increased during the growing season with 0.45-2.15× multiplication rates recorded in Adana, South East Mediterranean Region of Turkey. Similar findings on population increase were obtained in Australia with P. thornei in a spring wheat environment, with the peak from October to January in the growing season (Pattison and Fisher, 1993). The results from the present study are in agreement with these earlier results; the population increase of both RLN species is similar and was related to increases in soil temperature that peaked in June and July in soil at 23-25 °C. Once the wheat plants were mature in July, the populations of *Pratylenchus* spp. decreased in the vicinity of the roots, presumably due to their downward movement in the soil as soil temperatures increased over the summer period.

These observations confirm adaptation of the biology of CCN and RLN to the agro-ecological conditions in Turkey. Detailed knowledge of the biology and population dynamics of these cereal nematodes under natural conditions will be useful for development of the most effective integrated control strategies to maintain nematode populations below economic damage thresholds.

## ACKNOWLEDGEMENTS

The authors thank Mr. Bayram Erbas (CIMMYT, Turkey) and Mr. Aydın Ciftci (MARA) for collecting and processing of the soil samples and Dr. Jose Crossa (CIMMYT, Mexico) for statistical analysis of the data. Use of the Ankara University, Faculty of Agriculture field station in Haymana and field assistance of colleagues from MARA Ankara Central Field Crops Research Institute is also gratefully acknowledged. CIMMYT (International Maize and Wheat Improvement Center) and GDAR-MARA (General Directorate of Agricultural Research – Ministry of Agriculture and Rural Affairs) are thanked for financial and technical support for the study.

## LITERATURE CITED

- Abidou H., El-Ahmed A., Nicol J.M., Bolat N., Rivoal R. and Yahyaoui A., 2005. Occurence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologia Mediterranea*, 33: 1-18.
- Andersen S., 1961. Resistens mod Harveal Heterodera avenae. Meddelelse Nr. 68 fra den KG1. Veterinaer-og Landbohojskoles, Copenhagen, Denmark, 179 pp.
- Banyer R.J. and Fisher J.M., 1971. Seasonal variation in hatching of eggs of *Heterodera avenae*. Nematologica, 17: 225-236.
- Brown R.H., 1987. Control strategies in low-value crops. Pp 351-387. *In*: Principles and Practice of Nematode Control in Crops (Brown R.H. and Kerry B.R., eds). Academic Press, London, UK.
- Clarke A.J. and Perry R.N., 1977. Hatching of cyst nematodes. Nematologica, 23: 350-368.
- Elekcioglu I.H. and Gözel U., 1997. Effect of mixed populations of *Paratrophurus acristylus, Pratylenchus thornei* and *Paratylenchus* sp. (Nematoda: Tylenchida) on yield parameters of wheat in Turkey. *International Journal of Nematology*, 7,2: 212-220.
- Fenwick D.W., 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology*, 18: 155-172.
- Fortuner R., 1977. Pratylenchus thornei. CIH Descriptions of Plant Parasitic Nematodes, Set 7, No. 93. Commonwealth Institute of Helminthology, 103 Peter's Street, S. Albans, Herts., England.
- Franklin M.T., 1951. The cyst-forming species of *Heterodera*. Commonwealth Agricultural Bureau, Farnham Royal, England No. 21.
- Hooper D.J., 1986. Handling, fixing, staining and mounting nematodes. Pp. 5-30 *In*: Laboratory Methods for Work with Plant and Soil Nematodes. (Southey J.F., ed.). Her Majesty's Stationery Office, London, UK.
- McDonald A. and Nicol J.M., 2005. Nematode Parasites of Cereals. Pp. 131-191. *In:* Plant Parasitic Nematodes in Tropical and Subtropical Agriculture (Luc M., Sikora R. and Bridge J., eds). Wallingford, CABI Publishing, UK.
- Meagher J.W., 1970. Seasonal fluctuations in numbers of larvae of the cereal cyst nematode (*Heterodera avenae*) and of *Pratylenchus minyus* and *Tylenchorhyncus brevidens* in soil. *Nematologica*, 16: 333-347.
- Nicol J.M., 1996. The distribution, pathogenicity and population dynamics of *Pratylenchus thornei* on wheat in South Australia. *PhD Thesis*, University of Adelaide, Australia, 236 pp.
- Nicol J.M., Rivoal R., Taylor S. and Zaharieva M., 2003. Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: distribution, yield loss, use of host resistance and integration of molecular tools. *Nematol*ogy Monographs and Perspectives, 2: 233-251.
- Nicol J., Bolat N., Bagci A., Hekimhan H., Elekcioglu H., Tunali B., Yildirim A.F., Sahin E., Kaplan A., Yorgancilar A., Tulek A., Toktay H., Uçkun Z., Akar T., Yazar S., Gül-

- Pattison A.B. and Fisher J., 1993. Dynamics and distribution of *Pratylenchus thornei* under cereals. Pp. 16-19. *In*: Proceedings of the *Pratylenchus* workshop in 9<sup>th</sup> Biennial APPS Conference (Vanstone V.A., Taylor S.P. and Nicol J.M., eds). 8-9 July, Hobart, Australia.
- Rivoal R., 1982. Caractérisation de deux écotypes d'*Heterodera avenae* en France par leurs cycles et conditions thermiques d'éclosion. *Bulletin OEPP*, 12: 353-359.
- Rivoal R., 1986. Biology of *Heterodera avenae* Wollenweber in France. IV. Comparative study of the hatching cycles of two ecotypes after their transfer to different climatic conditions. *Revue de Nématologie*, 9: 405-410.
- Rivoal R. and Cook R., 1993. Nematode Pests of Cereals. Pp. 259-303. In: Plant Parasitic Nematodes in Temperate Agriculture (Evans K., Trudgill D.L. and Webster J.M., eds). CAB International, Wallingford, UK.
- Rivoal R. and Sarr E., 1987. Field experiments on *Heterodera* avenae in France and implications for winter wheat performance. Nematologica, 33: 460-479.
- Scholz U. and Sikora R.A., 2004. Hatching behavior of *Heterodera latipons* Franklin under Syrian agro-ecological conditions. *Nematology*, 6: 245-256.
- Seinhorst J.W., 1966. The relationship between population increase and population density in plant parasitic nematodes.I. Introduction and migratory nematodes. *Nematologica*, 12: 157-169.
- Stirling G., Nicol J. and Reay F., 1999. Advisory Services for Nematode Pests Operational Guidelines. RIRDC Publication No 99/ 41: 111 pp.
- Taylor S. and Vanstone V., 1996. Nematodes do not have to be the root of all crop problems. *Australian Grain, 6, 2*: 679.
- Townshend J.L. and Anderson R.V., 1976. Pratylenchus neglectus [ = P. minyus]. CIH Descriptions of Plant Parasitic Nematodes, Set 6, No. 82. Commonwealth Institute of Helminthology, 103 St. Peter's Street, St. Albans, Herts, England.
- Vanstone V.A. and Nicol J.M., 1993. Factors affecting pathogenicity and multiplication of *Pratylenchus neglectus* and *P. thornei* in inoculation experiments. Pp. 9-14. *In*: Proceedings of the *Pratylenchus* workshop in 9<sup>th</sup> Biennial APPS Conference (Vanstone V.A., Taylor S.P. and Nicol J.M., eds). 8-9 July, Hobart, Australia.
- Vanstone V.A., Rathjen A.J., Ware A.H. and Wheeler R.D., 1998. Relationship between root lesion nematodes (*Praty-lenchus neglectus* and *P. thornei*) and performance of wheat varieties. Australian Journal of Experimental Agriculture, 38: 181-188.
- Whitehead A.G. and Hemming J.R., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55: 25-38.
- Yorgancilar O, Sahin E., Belen S., Yorgancilar A., Bolat N. and Nicol J., 2005. Identification of cereal cyst nematodes (*Heterodera* spp.) using molecular techniques. Proceedings of 14th Biotechnology congress, 31 August-2 September, Osmangazi University Press, Eskisehir, Turkey, 496 pp.