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Vertical Distribution of Hop Cyst Nematode in Hop Gardens in Central Europe

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The genus *Heterodera* consists of 80 valid species which are distributed all over the world and they range from mono to polyphagous (Subotin et al., 2010). Several species are considered economically important (or quarantine), including *H. humuli* Filipjev, 1934. Previous studies on *H. humuli* or *H. humuli* as a vector of plant viruses were focused on its geographical distribution, biology, life cycle, and pathogenicity symptoms (Barbez, 1982; Lopez-Robles, 1995; Valdez et al., 1974). There were no reports of the vertical distribution of this species within the soil profile which is dependent on the large root system of hop plants. Mature hop plants have two types of roots: lateral spreading roots, 2-5 m long, that are wiry and extensively branched resulting in a fibrous layer in the top 20-30 cm of the soil, and vertical roots, 2-9 m long, that are fleshy, irregularly swollen, brittle and rarely branched. High population densities of *H. humuli* were found only in young, fibrous roots of hop plants (Mikhailyukov and Sigareva, 1981).

The objective of our investigation was to obtain information on the vertical distribution of the cysts, juveniles and males of *H. humuli* within the soil rhizosphere of hop plants. We hypothesized that the highest amount of cysts, juveniles and males of this nematode is located in the higher soil depth due to the presence of young root systems; however deep plowing, to a depth of 30-60 cm, could transfer the cysts deeper in the soil where they could remain as a residual source of infection.

The vertical distribution of hop cyst nematode was studied in 17 hop gardens situated in the Czech and Slovak Republic where the Žatecký poloraný červeňák, Sládek and Premiant hop cultivars susceptible to hop cyst nematode, are grown (Fig. 1). Ten soil composite samples were collected from the rhizosphere of hop plants from each locality from the depth of 0-20 and 20-40 cm in 2005, 2007 and 2008. Juveniles and males were isolated from 100 g of mixed soil by using Cobb flotation-sieving method, fixed in FAA and determined microscopically (Meyl, 1961), while the cysts were isolated using the flotation method from 200 g of dried soil sample (Sabová and Valocká, 1980). The number of collected cysts are shown in Table 1 and 2; the cysts were re-counted using 100 g of soil.

The number of cysts did not decrease with soil depth in all surveyed hop gardens (Table 1 and 2). Only in the hop root rhizosphere in eight of the hop gardens, a significantly lower (P=0.05) number of cyst was found in soil depth of 20-40 cm in comparison to 0-20 cm soil depth. In three of the hop gardens, cysts from even deeper soil depths were extracted but no significant differences were noted. Similar results were found with the vertical distribution of juveniles and males of *H. humuli*. A significantly higher number of juveniles was found in 0-20 cm soil depth in comparison to 20-40 cm in fourteen of the hop gardens, however, significantly higher number of males was found only in four of the hop gardens (P=0.05).

The study of nematode vertical distribution in the soil profile is difficult due to many interrelated factors including soil moisture, temperature, soil texture, height of water table, soil type, and depth of subsoil. However, the factors that influence nematode distribution most are the host roots presence, rooting patterns and root (Valdez, 1974) distribution. Nombela (1993) stated that vertical distribution of nematodes was related to the clay content in deep levels of soils, moisture content and host plant presence. The root system of host plants has probably great importance on vertical and spatial distribution of sedentary ectoparasitic cyst nematodes. For example, the potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida,* were found in high numbers in soil depth of 20-40 cm as in soil depth 0-20 cm (Whitehead, 1977). *Heterodera schachtii* cysts were numerous in the 60 cm soil depth and *H. goettingiana* in 20 cm soil depth (Whitehead, 1977). Been and Schomaker (2000) found 90% of PCN populations in the upper 35 cm of soil, however, several cysts were occasionally detected at depths of 80 cm and sometimes even deeper. However, in the cyst-forming nematodes, most of the specimens found in the soil are the mobile forms such as the second stage juveniles (J2) which can move independently in the soil profile in response to plant root growth or changes in soil temperature and humidity.

To sum up, the root distribution is probably the dominant factor which influences the distribution of plant parasitic nematodes in the soil although physical and anthropogenic factors also play an important role. Been and Schomaker (2000) stated that the spatial distribution of cysts within the soil profile is partially affected by agricultural practices, mainly by tillage.

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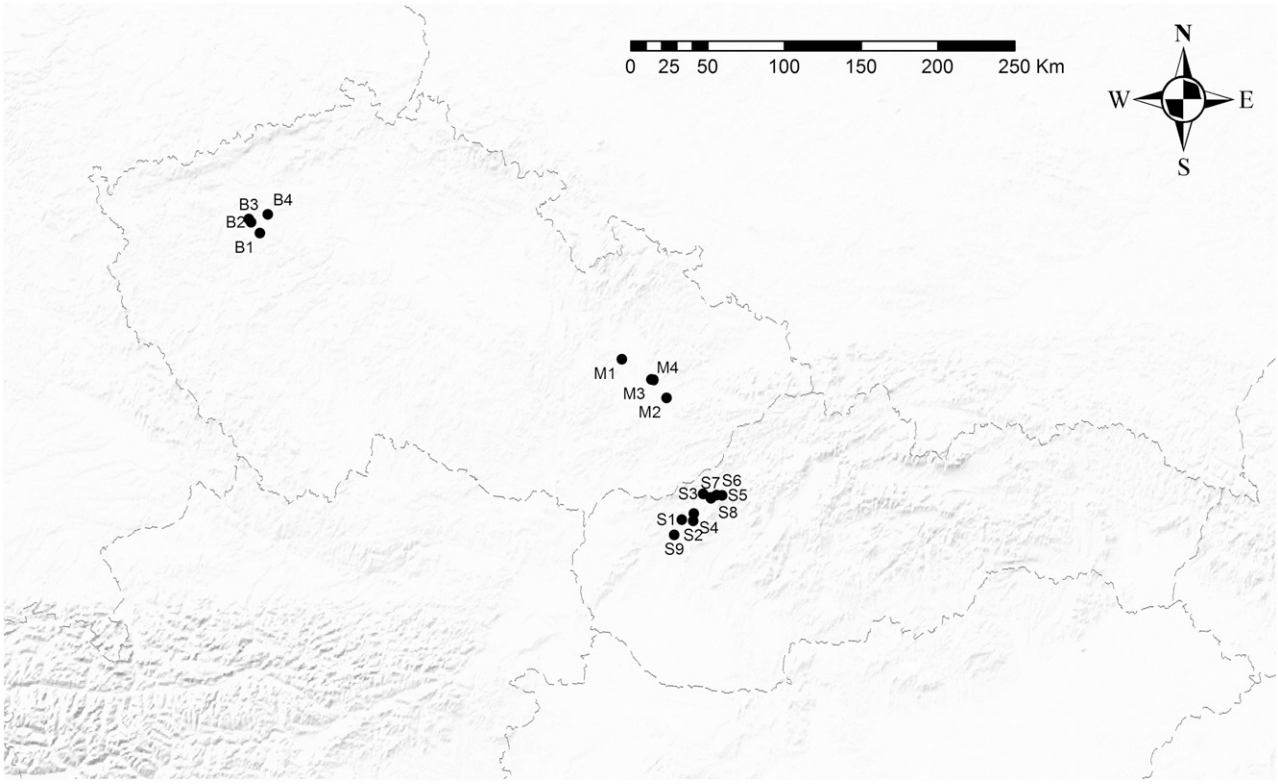

FIG. 1. Localities in Czech Republic (M1 – Senice na Hané, M2 – Domaželice, M3 – Tršice, M4 – Nelešovice, B1 – Lubná, B2 – Kněževes, B3 – Hořesedly, B4 – Třeboc); localities in Slovak Republic (S1 – Čachtice, S2 – Hôrka nad Váhom, S3 – Chocholná, S4 – Kočovce, S5 – Nemšová, S6 – Soblahov, S7 – Trenčianske Stankovce, S8 – Trenčianska Turná, S9 – Vrbové).

Boag and Neilson (1994) reported that cysts are usually distributed vertically in a random fashion in the top 20 cm in agricultural soils by cultivation. They reported that the depth to which samples are taken e.g. within 20 cm depth of the field soil would have little or no effect on the detection of population of PCN. Also it was reported that the vertical distribution of PCN cysts in the upper 25 cm of the soil was uniform and soil cultivation had no additional effect on the vertical distribution of the cysts and no consequences on the required depth of soil sampling (Whitehead, 1977).

The economic importance of *Heterodera* species increases in monocenoses of annual host plants or in permanent cultures such as hop gardens. However, there are several factors which can reduce nematode pathogenicity and reproduction in relation to agricultural practices. The number of recorded cysts, juveniles and males of *H. humuli* detected in the soil samples were relatively low within the surveyed hop gardens. The presumable reason is a periodical organic fertilization by cattle manure and deep tillage regimes. It has been confirmed in many studies that the organic amendments had suppressive effects on the development of plant parasitic nematodes including cyst nematodes (Renčo et al., 2010; Sasanelli, 2009). Periodical tillage together with soil organic amendments can also influence the population number of hop cyst nematodes and maintain them below threshold level (Rahman et al., 2007).

The results confirmed our hypothesis that deep autumn plowing may cause the transport of mature brown cysts from upper to deeper soil layers, where they can remain as a source of infection. The presence of juveniles and males in deeper soil layers was confirmed, as well. However, the distribution of fibrous roots of hop plants to depth of 30 cm, is also the main factor of occurrence of all developmental stages of *H. humuli* in both surveyed soil depths. On the other hand, a periodical deep cultivation

TABLE 1. The number of cysts, juveniles and males with soil profile of Czech hop gardens

	No. cysts		No. juveniles		No. males	
Localities	0-20	20-40	0-20	20-40	0-20	20-40
M1	81.5 a	60.2 a	59.5 a	5.5 b	7.0 a	3.0 a
M2	54.2 a	10.1 b	97.0 a	3.0 b	1.5 a	0.5 a
M3	4.2 a	6.0 a	17.0 a	1.5 b	1.0 -	0.0 -
M3	41.0 a	26.6 b	8.0 a	15.5 a	3.0 a	6.0 a
B1	10.1 a	7.3 a	61.0 a	6.0 b	3.0 -	0.0 -
B2	6.6 a	7.7 a	39.0 a	14.0 b	2.0 a	0.5 a
B3	1.2 a	1.6 a	9.5 a	1.0 b	0.0 -	0.0 -
B4	57.3 a	22.2 b	116.5 a	26.0 b	0.0 -	0.0 -

Each value is a mean of ten replications (n=10). Data marked by the same letters in each rows for number of cysts, juveniles and males are not statistically different according to Duncan's Multiple Range Test (P = 0.05).

TABLE 2. The number of cysts, juveniles and males with soil profile Slovak hop gardens

	No. cysts		No. juveniles		No. males	
Localities	0-20	20-40	0-20	20-40	0-20	20-40
S1	108.2 a	15.2 b	11.2 a	2.0 b	1.2 a	0.4 a
S2	18.5 a	17.1 a	5.6 a	1.6 b	0.4 a	0.2 a
S3	38.1 a	5.3 b	3.8 a	1.8 a	1.6 a	0.5 b
S4	69.3 a	27.2 b	9.6 a	2.2 b	3.5 a	0.6 b
S5	142.0 a	48.9 b	15.6 a	1.0 b	5.4 a	0.8 b
S6	47.2 a	24.1 a	12.4 a	1.8 b	0.6 a	0.6 a
S7	10.2 a	3.1 b	3.4 a	0.4 b	2.8 a	1.0 a
S8	18.9 a	15.3 a	5.8 a	0.6 b	0.2 a	0.4 a
S9	94.2 a	78.3 a	7.4 a	12.0 a	3.8 a	1.6 a

Each value is a mean of ten replications (n=10). Data marked by the same letters in each rows for number of cysts, juveniles and males are not statistically different according to Duncan's Multiple Range Test (P = 0.05).

and application of organic fertilizers are probably important factors of relatively low number of mature cysts found in all the surveyed hop gardens, where hop is grown in permanent monoculture for more than 20 years. However, further survey of deeper soil profile layers, i.e. 40-100 cm depth, in hop gardens should be conducted to understand the effect of soil cultivation and fibrous root development on the vertical distribution of *H. humuli* cysts.

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