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The influence of Western corn rootworm seed coating and granular insecticides on the seasonal fluctuations of soil nematode communities in a maize field

A. ČEREVKOVÁ[1], Ľ. CAGÁŇ[2]

[1]Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic, E-mail: *cerev@saske.sk*;
[2]Slovak Agricultural University, A. Hlinku 2, 94976 Nitra, Slovak Republic, E-mail: *ludovit.cagan@gmail.com*

Summary

Western corn rootworm, *Diabrotica virgifera virgifera*, is a pest that needs to be controlled with chemicals in the maize monoculture. The aim of this study was to examine the influence of insecticides on nematode communities in a maize field. Four soil treatments were used in this experiment: variant 1 – a granular application with tefluthrin (199.5 g a.i./ha); variant 2 – a granular application with clothianidin (110 g a.i./ha); variant 3 – a seed treatment with clothianidin (1.25 µg a.i/seed); and control – a maize field without insecticides.
During the investigated period, a total of 19 117 soil nematode individuals were captured and 9 orders, 33 genera and 37 species were identified. *Acrobeloides nanus, Cephalobus persegnis, Eucephalobus striatus* and *Basiria gracilis* were the dominant species, accounting for 48 % of the total number of individuals. The mean abundance and species diversity index were significantly lower for variant 2. Bacterial feeders were the dominant trophic group for all 4 variants. The numbers of nematodes in particular trophic groups (i.e., bacterial feeders, fungal feeders and omnivores) were significantly different between variant 2 and the control. The Σ Maturity index, Maturity index and Plant parasitic index did not show significant differences among the variants. The higher values of the Enrichment and Structure indices were observed in the first month of the investigation in all 4 variants. A cluster analysis showed that nematode species population densities were strongly affected by the date of soil sample collection and by the variants used in the experiment.

Keywords: Nematodes; *Diabrotica*; insecticides; soil environment

Introduction

Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is the most destructive pest of maize (*Zea mays* L.) in North America and began to successfully invade Central Europe in the early 1990s (Toepfer & Kuhlmann, 2004). Seed coating or granular insecticides are the usual methods of soil pest control in maize monoculture, and clothianidin and tefluthrin are often used to control this pest (Pilz *et al*., 2009).
Before the invasion of *D. v. virgifera* to Slovakia, the chemical control of maize soil pests was only used sporadically because of elaterid larvae. After 2004, the situation had completely changed. In the south of Slovakia the damage caused by *D. v. virgifera* reached more than 80 % (Cagáň, 2008) and the behaviour of farmers changed quickly. Farmers observed that they needed to use crop rotation or chemical control. However, many farms grew maize in almost 50 % of their area. Thus, chemical control of *D. v. virgifera* was an obligation. This issue became an absolutely new situation. Maize culture, which before was almost free from insecticides, started to endanger the environment.
Communities of nematodes are frequently used as suitable bioindicators based on their occurrence in all types of soil, a high abundance and the presence of all trophic groups.
Earlier studies on nematodes in maize throughout the world were focused mostly on plant-parasitic nematodes that feed on maize (Bernard *et al*., 2010; Bowen *et al*., 2008; Tylka *et al*., 2011) or the impact of crop rotation on nematode communities (Drake *et al*., 2010; Manachini *et al*., 2009; Rahman *et al*., 2007; Villenave *et al*., 2009). It was also mentioned that low infections of plant-parasitic nematodes may lead to greater availability of plant nutrients (Yeates *et al*., 1999). In Slovakia, the structure of nematode communities in maize was studied by Šály (1970) and Sabová *et al*. (1979), but information on the seasonal fluctuations of nematodes in maize is lacking. Similarly, data on the impact of *D. v. virgifera* insecticides on nematode populations in maize fields are insufficient. Therefore, the objective of this investigation was to obtain

information regarding the seasonal fluctuations of nematode populations in maize fields and to determine the impact of chemical treatments on nematode communities.

Materials and methods

Characteristic of locality and field trial

The field trial was conducted at Komoča (47.969, 18.022) in southwest Slovakia. The site was situated in a warm region with an average of 50 or more summer days annually and a warm and dry sub-region with a mild winter. An average daily temperature in January is higher than -3°C and an average daily temperature in July reaches 20 – 21°C. The mean annual precipitation total is 500-550 mm. The location is characterised by calcaric fluvisols associated with gleyic and arenic calcaric fluvisols from carbonate alluvial segments. Soil has a clayey texture (Miklós, 2002). The temperature during the period of investigation (May – September 2011) ranged from 16.7 – 21.6ºC and rainfall ranged from 15.4 – 83.7 mm.

The maize hybrid Realli CS (Caussade Semences) was used in the experiment.

The following variants were used in the trial:

Variant 1: a granular insecticide application into the rows during sowing – Force™ 1.5 G (Syngenta) tefluthrin 1.5 %, 199.5 g a.i./ha = 13.3 kg product/ha.

Variant 2: a granular insecticide application into the rows during sowing – Santana™ (Arysta LifeScience) clothianidin 1 % 110 g a.i./ha = 11 kg product/ha.

Variant 3: a commercially applied seed treatment – Poncho™ 600 FS (Bayer CropScience) clothianidin 104 ml/70000 seeds (per hectare) = 1.25 µg a.i/seed.

Control variant: a maize field without soil insecticides.

Maize was sown on April 27. Sampling was carried out during five months in 2011 on the following dates: May 4, June 8, July 6, August 3 and September 5.

Field trial included four variants randomly arranged in 5 repetitions. Plot size was 100 m^2. For each research variant, five bulked samples of soil (one per each plot) were used for investigating nematode communities. A bulked sample consisted of five sub-samples collected at a depth of 15 cm on the individual sampling dates.

Extraction and identification of nematodes

Nematodes were isolated from 100 g of mixed fresh soil samples using the Baermann method, fixed in FAA solution and evaluated on permanent glycerine slides (Southey, 1986). All isolated nematodes were determined to species level and juveniles were determined to the genus level using light microscope Nikon eclipse 90*i*.

Nematode community analysis

Nematode taxa were assigned to trophic groups according to Yeates *et al*. (1993). Community indices were calculated as follows: the Shannon-Weaver species diversity index (H'spp) (Shannon & Weaver, 1949); the Maturity index (MI) and Plant parasite index (PPI) proposed by Bongers (1990); ratio of PPI/MI proposed by Bongers and Korthals (1995); ratio of bacterial feeders and fungal feeders (B/F) proposed by Wasilewska (1997); the Enrichment index

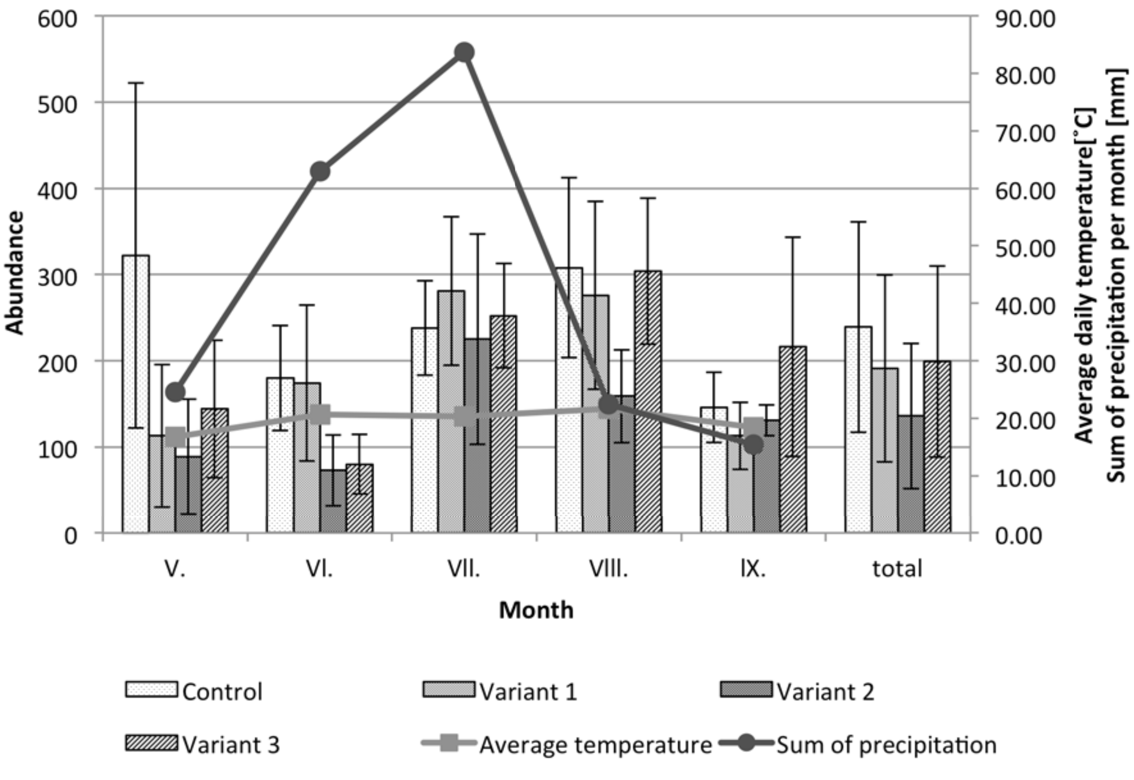

Fig. 1. Abundance of nematodes in 100 g of soil, calculated for each month from May to September (*n*=5) and for each variant in total (*n*= 25) at the locality Komoča (Slovakia) during 2011. Variant 1 includes the treatment with tefluthrin and variants 2 and 3 include granular and seed treatments with clothianidin.

Table 1. A check-list of nematode species, their mean abundance in each month from May to September ($n = 5$) and the total mean abundance of the variants ($n = 25$).
TG - allocation of species to trophic groups BF - bacterial feeders, FF - fungal feeders, O - omnivores, P - predators, RFF - root-fungal feeders and PP - plant parasites.

		Control						Variant 1						Variant 2						Variant 3					
TG	**Nematode species**	**V.**	**VI.**	**VII.**	**VIII.**	**IX.**	**total**	**V.**	**VI.**	**VII.**	**VIII.**	**IX.**	**total**	**V.**	**VI.**	**VII.**	**VIII.**	**IX.**	**total**	**V.**	**VI.**	**VII.**	**VIII.**	**IX.**	**total**
	ARAEOLAIMIDA																								
BF	*Plectus longicaudatus*	1.2	-	-	-	-	**0.24**	-	-	-	-	-	**-**	0.4	-	1.2	-	0.2	**0.36**	0.4	0.4	1	0.4	-	**0.44**
BF	*Plectus parvus*	3.2	2.8	1.4	1.4	-	**1.76**	1.2	0.6	2.2	1.2	-	**1.04**	0.4	-	0.6	1.2	0.2	**0.48**	4.2	-	1.4	-	0.2	**1.16**
	RHABDITIDA																								
BF	*Cephalobus persegnis*	2.2	16.6	33	44	28.8	**24.92**	3.4	11.8	29.4	22.4	19.2	**17.24**	4	7.6	24.4	25.8	18.6	**16.08**	9.4	6.6	36.8	59.6	45.8	**31.64**
BF	*Eucephalobus striatus*	7.2	35.8	27.8	30.6	15.6	**23.4**	2	9	23.4	19	5.4	**11.76**	3.6	2	10.2	14.2	10	**8**	18.4	3.4	16.6	36.6	17.8	**18.56**
BF	*Acrobeloides nanus*	1	32.4	64.8	67.8	28.2	**38.84**	4.2	24.2	78.6	87.2	38.2	**46.48**	5	14.4	74.8	31.4	40.6	**33.24**	13.8	22.8	50	91.4	48.2	**45.24**
BF	*Chiloplacus propinquus*	1.4	1.2	-	0.4	0.4	**0.68**	-	-	0.6	0.4	-	**0.2**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
BF	*Cervidellus vexilliger*	2.8	0.8	1.8	0.4	1	**1.36**	0.2	-	0.6	-	-	**0.16**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
BF	*Panagrolaimus rigidus*	4	-	0.6	1	0.2	**1.16**	0.4	-	-	0.4	-	**0.16**	0.2	-	-	0.2	-	**0.08**	0.4	-	-	-	-	**0.08**
BF	*Rhabditis spp.*	251.8	17.4	18.6	29.2	12.4	**65.88**	55	15.8	19.6	34.4	17.2	**28.4**	49.6	21	27.4	14.6	11.4	**24.8**	71	9.8	32	38	22.4	**34.64**
	APHELENCHIDA																								
FF	*Aphelenchus avenae*	3.6	8.6	3.2	5.2	-	**4.12**	0.4	3.6	4.2	1.6	1.2	**2.2**	1	10.6	2.6	1.6	2	**3.56**	-	5.8	3.6	0.8	1	**2.24**
FF	*Aphelenchoides composticola*	2	4.4	8.8	10.2	6.2	**6.32**	6	14	8	13.8	1.8	**8.72**	2.4	3.2	3	3.4	4	**3.2**	1.2	2.6	8.2	9.4	11	**6.48**
FF	*Aphelenchoides minimus*	5.2	11.4	8.6	17.8	12	**11**	2.4	7.8	23	9	6.4	**9.72**	0.4	4.4	13	8.6	17	**8.68**	3.2	5	8.4	10	19.6	**9.24**
FF	*Aphelenchoides ritzemabosi*	3	9.2	9	13.4	4	**7.72**	3.8	7	7.6	13	-	**6.28**	-	-	3	-	0.2	**0.64**	-	-	-	-	-	**-**
	TYLENCHIDA																								
RFF	*Aglenchus agricola*	1.6	-	-	-	-	**0.32**	-	-	-	-	-	**-**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
RFF	*Filenchus vulgaris*	5.6	11.4	24.6	17.2	9.2	**13.6**	6.8	7.4	21.2	8.2	1	**8.92**	5	2	10.4	7.8	4.8	**6**	5.4	2.8	22	14.4	14.4	**11.8**
RFF	*Basiria gracilis*	-	-	-	19.4	2.6	**4.4**	-	40.8	14.4	36.8	3.4	**19.08**	2.2	1.4	27.8	14.6	14.2	**12.04**	2.2	13	45.2	16.6	10.4	**17.48**
RFF	*Malenchus exiguus*	-	0.2	-	-	-	**0.04**	0.4	-	-	-	2.6	**0.6**	-	-	-	-	-	**-**	-	0.6	-	-	2.6	**0.64**
PP	*Bitylenchus dubius*	2.2	10	8.8	12.8	9.8	**8.72**	2.6	9	9.8	5.8	3.8	**6.2**	1.2	1	9.8	10.8	2.4	**5.04**	1	3	9.6	9.4	3	**5.2**
PP	*Pratylenchus pratensis*	0.2	-	-	1.6	0.6	**0.48**	-	0.8	0.2	1.2	0.4	**0.52**	0.4	-	-	-	0.4	**0.16**	-	-	-	-	-	**-**
PP	*Pratylenchoides crenicauda*	0.2	1.2	-	-	-	**0.28**	1.6	0.6	-	-	-	**0.44**	0.2	0.2	-	-	-	**0.08**	-	-	-	-	-	**-**
PP	*Helicotylenchus digonicus*	0.2	-	-	-	-	**0.04**	-	0.6	0.2	-	0.2	**0.2**	-	-	-	-	-	**-**	1	0.4	1.2	0.4	-	**0.6**
PP	*Paratylenchus sp.*	-	-	-	-	-	**-**	-	-	-	-	-	**-**	-	-	0.2	-	-	**0.04**	0.6	-	1	0.4	-	**0.4**
	ENOPLIDA																								
BF	*Prismatolaimus dolichurus*	-	-	-	0.4	4	**0.88**	0.4	-	4.8	2.6	4	**2.36**	-	-	-	2.2	1	**0.64**	-	-	0.2	0.2	0.2	**0.12**

	ALAIMIDA																								
BF	*Alaimus primitivus*	1.2	0.4	1.4	2.4	0.4	**1.16**	-	0.2	3	1	0.2	**0.88**	0.2	0.6	1.8	1	0.4	**0.8**	-	0.2	1.4	1.4	3.2	**1.24**
	DIPHTHEROPHORIDA																								
O	*Diphtherophora communis*	-	-	-	0.2	-	**0.04**	-	-	-	-	-	**-**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
PP	*Trichodorus primitivus*	-	-	0.6	0.4	-	**0.2**	-	-	-	0.2	-	**0.04**	-	0.2	0.6	2.6	-	**0.68**	-	0.2	0.6	0.6	-	**0.28**
	MONONCHIDA																								
P	*Coomansus parvus*	0.2	-	-	-	-	**0.04**	3.2	-	-	-	-	**0.64**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
P	*Mylonchulus brachyuris*	7	1.8	3.4	4.4	2.6	**3.84**	1.6	1	3.2	1.6	2.6	**2**	2.4	0.2	1.4	5	1.6	**2.12**	3	0.2	9.4	4.8	8.6	**5.2**
P	*Prionchulus muscorum*	2	-	-	0.4	2.4	**0.96**	-	-	-	1.2	-	**0.24**	0.8	-	3.6	8.2	0.6	**2.64**	0.8	-	0.2	-	-	**0.2**
	DORYLAIMIDA																								
O	*Mesodorylaimus bastiani*	-	-	0.2	-	-	**0.04**	0.2	-	-	0.2	-	**0.08**	-	-	0.2	-	-	**0.04**	-	-	-	0.6	-	**0.12**
O	*Mesodorylaimus centrocercus*	-	0.8	-	-	-	**0.16**	-	-	-	-	-	**-**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
O	*Thonus ettersbergensis*	7.6	3.6	4.8	10.2	3.2	**5.88**	2.2	2	13.8	6.6	0.4	**5**	-	0.6	2	-	-	**0.52**	-	-	-	-	-	**-**
O	*Dorydorella pratensis*	2	6.4	7.2	11.8	0.6	**5.6**	5.8	10	1.2	-	0.6	**3.52**	9	0.8	1.8	2	-	**2.72**	0.8	0.2	0.8	1.4	1.8	**1**
O	*Eudorylaimus carteri*	0.2	1	0.2	0.8	0.2	**0.48**	9.4	1.2	6	6.2	2.2	**5**	-	2.8	4.8	2.8	0.6	**2.2**	5	3.2	1.6	6.2	3.8	**3.96**
O	*Eudorylaimus iners*	0.4	-	-	-	-	**0.08**	-	-	0.4	-	-	**0.08**	-	-	-	-	-	**-**	-	-	-	-	-	**-**
O	*Aporcelaimellus obtusicaudatus*	2.2	1.8	0.8	2.8	1.2	**1.76**	0.2	0.8	0.4	2.4	1.6	**1.08**	0.6	-	0.6	1	1	**0.64**	0.6	0.2	0.6	1.4	1.8	**0.92**
O	*Enchodelus macrodorus*	-	-	1	0.4	0.6	**0.4**	-	-	-	-	0.4	**0.08**	-	-	-	-	-	**-**	1.2	-	-	-	-	**0.24**
PP	*Doryllium zeelandicum*	0.6	0.4	7	1.6	-	1.92	-	5.4	4.8	-	-	**2.04**	-	-	-	-	-	**-**	-	-	-	-	-	**-**

(EI), the Structure index (SI) and the Channel index (CI) according to Ferris *et al.* (2001) with weightings of nematode taxa as suggested by the authors. The weightings for taxa not included in this study were derived from the c-p values by Bongers (1990). The abundance of nematodes, H'spp, abundance of nematodes in trophic groups, ecological indexes and all other indices were calculated as the mean.

Data analysis
An analysis of variance was used for statistical analysis; the means were compared by the Tukey's HSD test (p > 0.05). Five repetitions from each variant were used in calculation of difference among four variants. Data from each date and total data were evaluated separately. Programme "Statistica" was used for the calculations (Tables 2 and 3). Cluster analysis was performed by using Ward's method of Euclidean distances (StatSoft, 2001).

Results

Taxonomical evaluation of nematode communities
A total of 37 nematode species and 33 genera, including 7 species of plant parasites, were identified in the maize field during the investigated period. The number of identified species (36) and genera (32) in the control was higher than in variants 1 – 3 (25 – 32 species or 24 – 30 genera) (Table 1). The total abundance of nematodes was significantly lower in variant 2 (Fig. 1). The highest total abundance of nematodes in the control was mostly caused by a high abundance of *Rhabditis* spp. in May (also confirmed statistically by an ANOVA and Tukey multiple range test (p = 0.05). Some species were observed abundantly in the control but occurred with low abundance or did not occur at all in variants with chemical applications. A higher abundance of nematodes in the control and variant 1 were caused by higher abundance of the species *Aphelenchoides ritzemabosi, Doryllium zeelandicum* and *Thonus ettersbergensis*, in variants 2 and 3, these nematode species occurred in low abundance. On the other hand, the abundance of *Basiria gracilis* was significantly higher in variants 1 – 3 compared to the control (Table 1). The abundance of nematodes was markedly changed during the season in all variants and higher changes were observed in variant 3. In the second month after the granular application, variant 3 contained only 80 nematode individuals, however, in August (approximately four months after the granular application), abundance increased to 304 individuals in 100 g of soil. The least amount of change in abundance during the season was observed in the control and in variant 1 (Fig. 1). Out of a total number of identified nematode species 7 species were obligate plant parasite nematodes. Only two plant parasite species were identified in all investigated variants: *Bitylenchus dubius* and *Trichodorus primitivus* (Table 1).

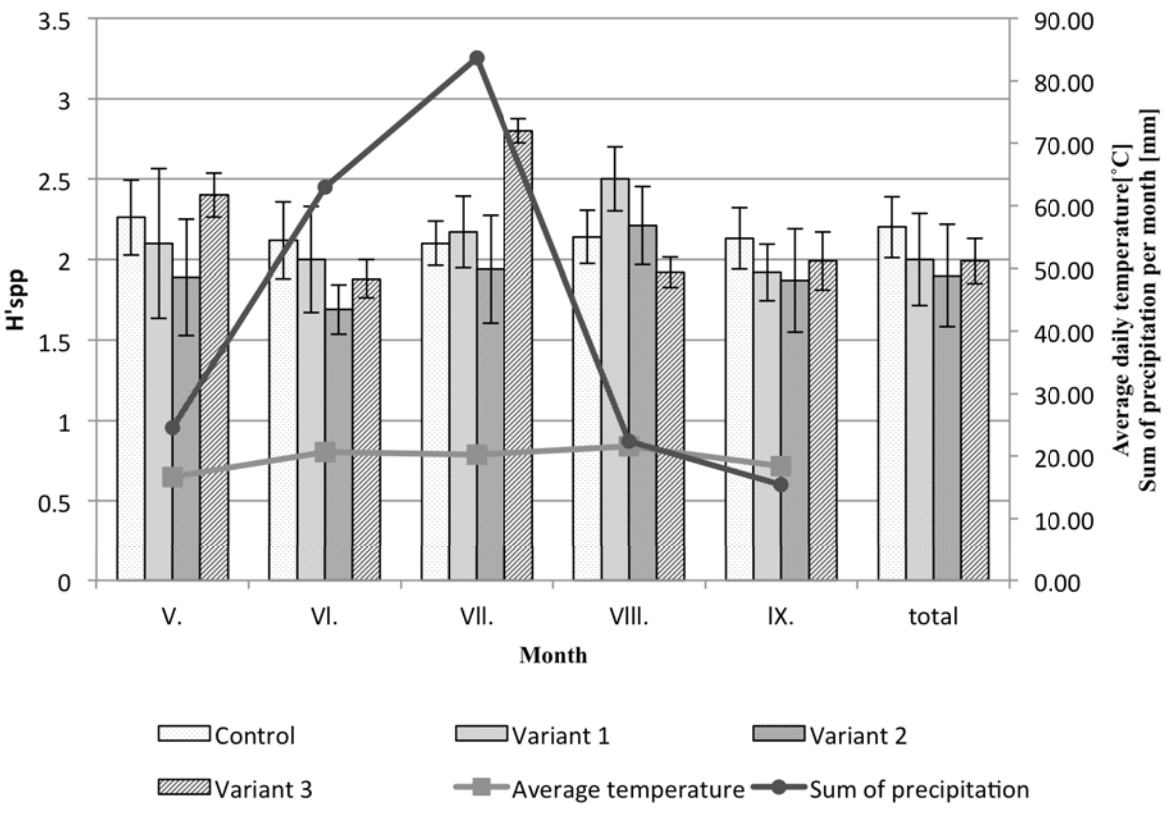

Fig. 2. Shannon-Weaver Diversity Index H'spp, calculated for each month from May to September (n=5) and for each variant in total (n = 25). Locality Komoča (Slovakia) during 2011. Variant 1 includes the treatment with tefluthrin and variants 2 and 3 include granular and seed treatments with clothianidin.

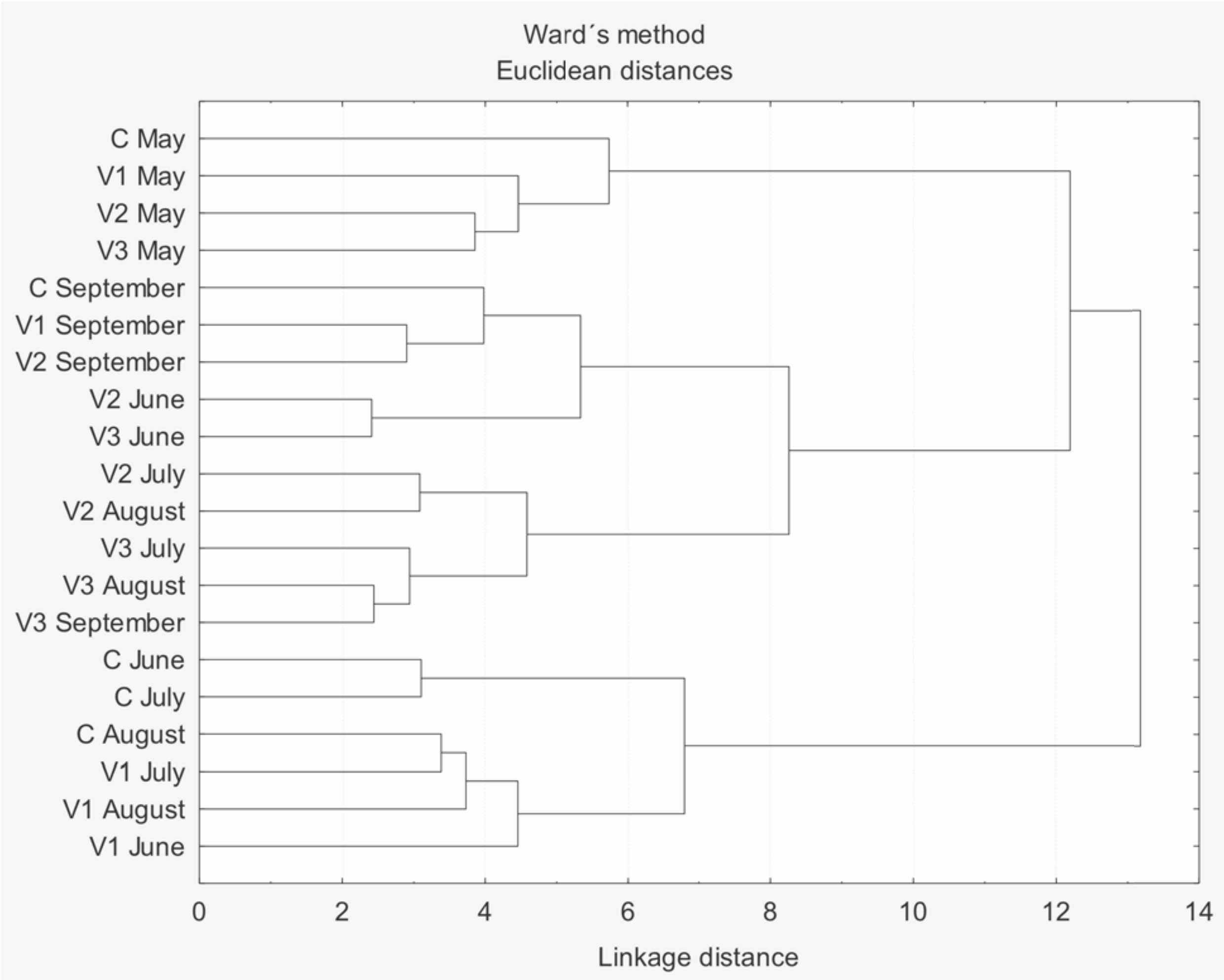

Fig. 3. Cluster analysis of nematode species population densities in a maize field during May to September 2011 in four soil treatments: V1 - variant 1; V2 - variant 2; V3 - variant 3; C - control. Variant 1 includes the treatment with tefluthrin and variants 2 and 3 include granular and seed treatments with clothianidin. Data log(x+1) where x = species population density. Locality Komoča, Slovakia.

Diversity of nematode species

Values for the diversity index H'spp indicated different species structures of nematode communities. The highest H'spp was recorded in the control. On the other hand, the lowest total H'spp was observed in variant 2, where the significantly lowest total abundance was also observed. When the nematode abundance and the H'spp in variant 2 were compared during season, the lowest values were observed in the second month of the evaluation (Fig. 2).

The cluster analysis of species population densities produced three main clusters: the upper cluster consisted of samples of all four variants collected in May; the lower cluster consisted of samples of the control and variant 1 sampled from June to August. The middle cluster contained two sub-clusters, which consisted of samples of variants 2 and 3 (except for two samples of the control and variant 1 that were collected in September) (Fig. 3).

Ecological evaluation of nematode communities based on trophic groups and ecological indices

Nematode communities were represented by all trophic groups. Bacterial feeders were the dominant trophic groups in all variants, with the lowest significant total abundance in variant 2 (Table 2). Similarly, the lowest total abundance of fungal feeders was observed in variant 2 and the lowest total abundance was observed in omnivores in variants 2 and 3 compared to the control. The change in abundance of plant parasites, root-fungal feeders and predators was small during May-September and significant differences were not observed between variants.

The calculated ecological indices ΣMI, MI, PPI and ratio PPI/MI values, were without significant differences in the variants (Table 3). The values of ratio B/F and CI fluctuated across all sampling dates and variants, but no significant differences in these values were observed except for the CI in variant 2 in June. A significant sampling date effect was observed in the values of the EI and SI. In May, a higher value of the EI and SI was observed in all variants and was caused mainly by a higher abundance of *Rhabditis* spp. and a lower abundance of *Acrobeloides nanus*, respectively. Using the EI and SI, most samples were characterised as containing a degraded soil food web with high C:N ratios and the decomposition channels were provided by fungi.

Table 2. Mean abundance of nematodes from 100 g of soil in trophic groups in the four different variants. From those, variant 1 includes the treatment with tefluthrin and variants 2 and 3 include granular and seed treatments with clothianidin. BF - bacterial feeders, FF - fungal feeders, O - omnivores, P - predators, RFF - root-fungal feeders, PP - plant parasites, calculated for each month from May to September ($n = 5$) and for each variant in total ($n = 25$). For details see Material and methods.

	Control						Variant 1						Variant 2						Variant 3					
	V.	VI.	VII.	VIII.	IX.	total	V.	VI.	VII.	VIII.	IX.	total	V.	VI.	VII.	VIII.	IX.	total	V.	VI.	VII.	VIII.	IX.	total
BF	276^{a}	107.4^{a}	149.4^{a}	177.6^{ab}	91.0^{a}	**160.3^{a}**	66.8^{b}	61.6^{b}	162.2^{a}	168.6^{ab}	84.2^{a}	**108.7^{ab}**	63.4^{b}	45.6^{b}	140.4^{a}	90.6^{b}	82.4^{a}	**84.5^{b}**	117.6^{ab}	43.2^{b}	139.4^{a}	227.6^{a}	137.8^{a}	**133.1^{ab}**
FF	14.4^{a}	34.0^{a}	36.6^{a}	48.2^{a}	22.2^{a}	**31.1^{a}**	12.6^{a}	37.8^{a}	47.6^{a}	37.4^{ab}	9.4^{a}	**29.0^{ab}**	3.8^{a}	18.2^{a}	21.6^{a}	13.6^{b}	23.2^{a}	**16.1^{b}**	4.4^{a}	13.4^{a}	20.2^{a}	20.2^{ab}	31.6^{a}	**18.0^{ab}**
O	12.4^{a}	13.6^{a}	14.2^{ab}	26.2^{a}	5.8^{a}	**14.4^{a}**	17.8^{a}	14.0^{a}	21.8^{b}	15.4^{ab}	5.2^{a}	**14.8^{a}**	9.6^{a}	4.2^{a}	9.4^{ab}	5.8^{b}	1.6^{a}	**6.1^{b}**	7.6^{a}	3.6^{a}	3.0^{a}	9.6^{ab}	7.4^{a}	**6.2^{b}**
P	9.2^{a}	1.8^{a}	3.4^{a}	4.8^{ab}	5.0^{a}	**4.8^{a}**	4.8^{a}	1.0^{a}	3.2^{a}	2.8^{a}	2.6^{a}	**2.9^{a}**	3.2^{a}	0.2^{a}	5.0^{a}	13.2^{b}	2.2^{a}	**4.8^{a}**	3.8^{a}	0.2^{a}	9.6^{a}	4.8^{ab}	8.6^{a}	**5.4^{a}**
RFF	7.2^{a}	11.6^{a}	24.6^{a}	36.6^{a}	11.8^{a}	**18.4^{a}**	7.2^{a}	48.2^{a}	35.6^{ab}	45.0^{a}	7.0^{a}	**28.6^{a}**	7.2^{a}	3.4^{a}	38.2^{ab}	22.4^{a}	19.0^{ab}	**18.0^{a}**	7.6^{a}	16.4^{a}	67.2^{b}	31.0^{a}	27.4^{b}	**30.0^{a}**
PP	2.8^{a}	11.2^{a}	9.4^{a}	14.8^{a}	10.4^{a}	**9.7^{a}**	4.2^{a}	11.0^{a}	10.2^{a}	7.2^{a}	4.4^{a}	**7.4^{a}**	1.8^{a}	1.4^{a}	10.6^{a}	13.4^{a}	2.8^{a}	**6.0^{a}**	2.6^{a}	3.6^{a}	12.4^{a}	10.8^{a}	3.0^{a}	**6.5^{a}**

Each four means in rows calculated for each month and for each variant in total followed by the same letter are not significantly different (ANOVA, Tukey's HSD test, $p > 0.05$).

Table 3. Ecological evaluation of nematode community structure in the four different variants. From those, variant 1 includes the treatment with tefluthrin and variants 2 and 3 include granular and seed treatments with clothianidin. ΣMI - Sum Maturity Index, MI - Maturity Index, PPI - Plant Parasitic Index, ratio of PPI/MI; B/F - ratio of bacterial feeders and fungal feeders; CI - Channel Index; EI - Enrichment Index ; SI - Structure Index. All indexes are calculated for each month from May to September ($n = 5$) and for each variant in total ($n = 25$). For details see Material and methods.

	Control						Variant 1						Variant 2						Variant 3					
	V.	VI.	VII.	VIII.	IX.	total	V.	VI.	VII.	VIII.	IX.	total	V.	VI.	VII.	VIII.	IX.	total	V.	VI.	VII.	VIII.	IX.	total
Σ MI	1.62^{a}	2.11^{a}	2.19^{bc}	2.18^{a}	2.11^{a}	**2.0^{a}**	2.23^{a}	2.28^{a}	2.26^{c}	2.09^{a}	2.08^{a}	**2.2^{a}**	2.05^{a}	1.92^{a}	2.08^{ab}	2.23^{a}	1.98^{a}	**2.1^{a}**	1.8^{a}	2.06^{a}	2.02^{a}	2.0^{a}	2.1^{a}	**2.0^{a}**
MI	1.63^{a}	2.13^{a}	2.22^{bc}	2.23^{a}	2.13^{a}	**2.1^{a}**	2.21^{a}	2.34^{a}	2.31^{c}	2.12^{a}	2.1^{a}	**2.2^{a}**	2.08^{a}	1.9^{a}	2.1^{ab}	2.28^{a}	1.97^{a}	**2.1^{a}**	1.79^{a}	2.07^{a}	2.01^{a}	2.0^{a}	2.13^{a}	**2.0^{a}**
PPI	1.61^{a}	2.0^{a}	2.03^{a}	2.02^{a}	2.0^{a}	**1.9^{a}**	2.0^{a}	2.02^{a}	2.02^{a}	2.0^{a}	2.02^{a}	**2.0^{a}**	2.0^{a}	2.1^{a}	2.02^{a}	2.07^{a}	2.0^{a}	**2.0^{a}**	2.07^{a}	2.06^{a}	2.03^{a}	2.0^{a}	2.0^{a}	**2.0^{a}**
PPI/MI	1.01^{a}	0.94^{ab}	0.92^{ab}	0.91^{a}	0.94^{a}	**0.9^{a}**	0.97^{a}	0.87^{b}	0.87^{a}	0.95^{a}	0.97^{a}	**0.9^{a}**	1.02^{a}	1.15^{a}	0.96^{ab}	0.91^{a}	1.02^{a}	**1.0^{a}**	1.2^{a}	1.01^{ab}	1.01^{b}	1.0^{a}	0.94^{a}	**1.0^{a}**
B/F	27.34^{a}	3.65^{a}	4.85^{a}	3.67^{a}	6.99^{a}	**9.3^{a}**	5.68^{a}	1.73^{a}	3.88^{a}	5.87^{a}	11.28^{a}	**5.7^{a}**	12.5^{a}	19.6^{a}	8.7^{a}	9.06^{a}	4.98^{a}	**10.4^{a}**	14.24^{a}	6.11^{a}	7.92^{a}	15.2^{a}	6.77^{a}	**9.7^{a}**
CI	2.48^{a}	11.1^{b}	6.2^{ab}	8.3^{ab}	7.1^{ab}	**7.0^{a}**	5.4^{a}	13.6^{b}	8.1^{ab}	6.6^{ab}	3.3^{a}	**7.4^{a}**	1.8^{a}	6.9^{a}	3.6^{a}	5.0^{a}	7.8^{a}	**5.0^{a}**	1.8^{a}	7.6^{b}	3.9^{ab}	2.5^{a}	5.1^{ab}	**4.2^{a}**
EI	93.6^{a}	45.7^{b}	39.7^{b}	45.5^{b}	41.2^{b}	**53.1^{a}**	89.4^{a}	53.9^{b}	40.4^{b}	51.0^{b}	44.6^{b}	**55.9^{a}**	82.0^{a}	75.4^{a}	38.6^{b}	46.0^{b}	41.6^{b}	**56.6^{a}**	82.5^{a}	48.9^{b}	52.2^{b}	44.2^{b}	37.2^{b}	**53.0^{a}**
SI	70.8^{a}	39.3^{b}	45.4^{b}	50.3^{ab}	38.7^{b}	**48.9^{ab}**	79.1^{a}	59.6^{ab}	51.2^{ab}	41.0^{b}	42.0^{b}	**54.6^{b}**	77.7^{a}	39.5^{bc}	37.7^{bc}	53.3^{ab}	19.2^{c}	**45.5^{ab}**	59.0^{a}	34.3^{a}	34.4^{a}	28.3^{a}	34.4^{a}	**38.1^{a}**

Each four means in rows calculated for each month and for each variant in total followed by the same letter are not significantly different (ANOVA, Tukey's HSD test, $p > 0.05$).

Discussion

Our study was conducted in a maize field to determine the impact of the season and chemical treatment on nematode communities. The nematode community was investigated for species composition, trophic structure and biodiversity. The findings from a control and three different chemically treated fields of corn were compared. A total of 37 nematode species and 33 genera were identified during the investigated period. This number of species and genera was higher than the number observed by Šály (1970) in maize fields in Slovakia (only 16 species). On the other hand, Landi and Manachini (2005) observed 45 genera in maize fields in Italy.

The total abundance was the highest in the control, mostly caused by a high abundance of *Rhabditis* in May. These results agree with many authors who recorded higher abundances of nematodes in soils without chemical treatment (JingNan *et al*., 2010; Waliyar *et al*., 1992; Zhou *et al*., 2008) or decreased nematode abundance and diversity with intense land cultivation (Kimenju *et al*., 2009). In contrast, Brmež *et al*. (2007) observed higher nematode abundance but a lower number of genera in the agricultural treatments than in the treatment without human investigation. It was also found that nematode species were present in greater numbers on fertilised soils (McIntosh *et al*., 1999).

In the control and variant 1 (tefluthrin), a higher abundance of the species *Aphelenchoides ritzemabosi, Doryllium zeelandicum* and *Thonus ettersbergensis* was observed. However, in variants 2 and 3 (clothianidin), these nematode species occurred in low abundance. It is important to note that the mode of action of tefluthrin and clothianidin is different. Tefluthrin is not transported in the plant from the roots to the leaves, but clothianidin is a systemic insecticide (Bewley *et al*., 2006; Schmuck & Keppler, 2003). However, the abundance of *Basiria gracilis* was significantly higher in variants with an application of chemicals (variants 1 – 3) compared to the control. We do not have an explanation for this result. *B. gracilis* was observed in 7 samples (from 25) in the control, in 17 samples in variant 1 and in 25 samples in both variants 2 and 3.

The decrease in nematode diversity was assessed using the diversity index H'spp and was used to reflect the underlying changes in physical, chemical and biological properties of the soil environment. In all examined variants, nematode diversity was low with a mean H'spp value of no more than 2.2 (control). Landi and Manachini (2005) observed a higher level of disturbance in eight maize fields in Italy, where the H'spp value was only 1.08. A comparison of nematode abundance and the H'spp in variant 2 revealed the lowest values in the second month of the evaluation.

The dominant trophic group was the bacterial feeders, and in particular, *Rhabditis* was the most abundant and, often, the most dominant species. Venette and Ferris (1997) observed an increase of bacterial feeders at higher temperatures. Similar results were observed in maize by Landi and Manachini (2005) where the genus of bacterial feeders *Rhabditis* and *Acrobeloides* together with plant parasites *Pratylenchus* and *Helicotylenchus* made up more than 70 % of the total nematode abundance collected. Similarly, the dominant trophic group of bacterial feeders, followed by the plant parasites, was observed by Manachini and Lozzia (2002) in a Bt maize crop and by Brmež *et al*. (2007) in soils with agricultural treatments. In contrast, Ou *et al*. (2005) observed bacterial feeders to be the most abundant group in paddy fields, while plant parasites were observed to be the most abundant group in maize fields. Kimenju *et al*. (2009) observed a predominance of plant parasitic nematodes in soils under agricultural production, while fungal feeder nematodes dominated in forested land.

Our results show that in the case of bacterial and fungal feeders, only variant 2 showed significant effects of nematode abundance in these trophic groups. It was observed that treatments in variants 2 and 3 significantly influenced omnivore nematode abundance.

In almost all variants, high densities of the obligate parasite *Bitylenchus dubius* and root-fungal feeders *Filenchus vulgaris* were observed. In particular, *B. dubius* is potentially harmful for maize plants in latitudes of temperate climatic zone. *B. dubius* frequently occurs in arable soil with various plants, e.g., cereals (Sabová *et al*., 1979; Šály, 1976) and other plants in arable soils (Šály, 1970, 1983). Williams and Beane (1984) consider this species a dangerous maize pest in England. Landi and Manachini (2005) and Khan *et al*. (2009) observed genera *Pratylenchus* and *Helicotylenchus* as dangerous plant parasites of maize. These genera were also recorded in our variants but with low abundance.

The Σ Maturity index, Maturity index, Plant parasitic index and ratio PPI/MI values did not show statistically significant differences among the treatments, although their trends served as effective tools. In agricultural soils regularly cultivated and fertilised by organic manures (Lišková & Renčo, 2007), recorded MI values are similar to those in maize crops with a dominance of bacterial feeder nematodes. This effect may be caused by the addition of organic matter that improves microbial activity in the soil.

The ratio of B/F is proposed to be an indicator of decomposition pathways. The Channel index (CI) improves the resolution of B/F and indicates the predominant decomposition pathways using selected taxa (Ferris *et al*., 2001). In our study, significant differences were not observed for the values of B/F and CI among the sampling dates and variants except for the data from June. The highest values of EI and SI were observed in all variants in the first month of the experiment. Berkelmans *et al*. (2003) reported that a higher SI indicated a well-regulated and healthy ecosystem. Zhang *et al*. (2010) reported that soil nematode community structure could be influenced by agrochemical application and using SI is a useful indicator to assess the impact of agrochemicals on soil ecosystems.

The application of different chemical treatments for the control of *Diabrotica virgifera virgifera* in maize showed little impact on the nematofauna, affecting neither species composition nor biodiversity. The calculated ecological indexes ΣMI, MI, PPI and PPI/MI values, were without significant differences in the investigated variants (Table 3). Only variant 2 caused a significant change in abundance, H'spp and trophic group composition. Bacterial feeders, fungal feeders and omnivores were also less numerous in variant 2 compared to the control. Voeste *et al*. (2010) mentioned that mixtures with clothianidin show an impact on many nematode species. However, another source (e.g., FAO, Clothianidin, 2010) mentioned that even though clothianidin has a broad spectrum of activity against insects, it shows no efficacy against spider mites and nematodes. In our variant 2 it was used 110 g a.i./ha, in variant 3 it was used 1.25 µg a.i/seed (less than 1 g a.i./ha). It is probably the reason why only variant 2 caused a significant change in abundance of nematodes. It was confirmed that overuse of pesticides has an effect on the amount and relative abundance of soil nematodes (Zhou *et al*., 2008). The effect of tefluthrin was not significant, and this result is expected because this chemical is used in combination with entomopathogenic nematodes (Nishimatsu & Jackson, 1998; Schmuck & Keppler, 2003).

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