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CONTROL AND MANAGEMENT OF PLANT-PARASITIC NEMATODES IN INTEGRATED PRODUCTION OF GARDEN STRAWBERRIES (*FRAGARIA×ANANASSA* DUCHESNE EX ROZIER)

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*Arable soils are one of the most valuable natural resources and their long-term sustainable management is a determining factor in the integrated production of strawberries. It is well known that the current large-fruited garden strawberry (*Fragaria × ananassa *Duchesne ex Rozier) cultivars are more susceptible to many species of plant-parasitic nematodes and other plant pathogens. The aim of this study was to evaluate the effects of some cultural practices as potential methods for control of nematodes in the integrated production of strawberries. The investigation of the nematode populations was carried out in the region of western Balkan Mountains in Bulgaria (43°33'22.3"N 22°47'03.4"E), with cultivar 'Maya'. In the surveyed area, plant protection products were applied under an approved scheme complying with the requirements for integrated fruit production (IOBC, IFP). Nematode populations were identified and classified to trophic level. The following genera of plant-parasitic nematodes were identified:* Pratylenchus crenatus, P. neglectus, P. thornei, Tylenchorhynchus sp.*, and* Paratylenchus *spp. The density and species composition of plant parasitic nematodes were significantly reduced at the end of the study period comparing to the beginning of the study. From the results, it is clear that the integrated production can be defined as an economically feasible production of high quality fruits, giving priority to environmentally safe methods of pest control.*

Key words: *integrated pest control, Nematoda,* Paratylenchus*, plant parasitic nematodes,* Pratylenchus, Tylenchorhynchus*.*

INTRODUCTION

In recent years, there has been growing interest in the production of strawberries (*Fragaria* × *ananassa* Duchesne ex Rozier) in several regions of Bulgaria. Because strawberries are often offered directly to customers in the field, farmers are looking for alternatives to chemical pesticides to protect human health and the environment. Integrated fruit production (IFP, IOBC) is defined as a cost-efficient production of high quality fruit, giving priority to environmentally safe methods to minimise the adverse side effects of the use of agrochemicals. Arable soils are one of the most valuable natural resources and their long-term sustainable management is a determining factor in the integrated production of strawberries.

The cultivation of susceptible cultivars in local agricultural systems leads to accumulation of pests and diseases. It is well known that the current large-fruited garden strawberry cultivars have a low degree of resistance to many species of plant-parasitic nematodes and other plant pathogens. The current farming practices have contributed to rapid spread of nematodes in the soil, which increases problems with plant-parasitic nematodes, especially during the transition period from conventional to organic farming (Hallmann *et al*., 2007). Plant-parasitic nematodes are known to be pests of fruit and vegetable crops in Bulgaria (Samaliev and Stoyanov, 2007). Studies indicate a large number of nematode species that are associated with strawberry plants and affect strawberry growth. These studies found that soil contains mixed populations of 10 genera and 15 species, of which are phytopathologically important parasites of strawberry and some are also vectors of plant viruses (Samaliev and Mohamedova, 2011). The use of soil fumigation to manage plant-parasitic nematodes is becoming limited as older products are no longer available (Zasada *et al*. 2010;

Pisanello, 2014) and alternative products are not providing the same efficacy (d'Errico *et al*., 2010). The new methods of nematode control need to be highly effective and safe, with long nematicide effect and with favourable environmental and toxicological properties.

Our experiments were designed to assess the used strategies for integrated cultivation and control of other pests, which might be used to suppress plant parasitic nematode populations. The aim of this study was to evaluate the applied practices as potential methods for control and management of plant parasitic nematodes in the integrated production of strawberries.

MATERIALS AND METHODS

The study of the nematode populations was carried out in the region of western Balkan Mountains in Bulgaria (43°33'22.3"N 22°47'03.4"E) during the vegetation period of 2013-2015. The studied strawberry plantation was established in 2012 on an area of 10 ha, with cultivar 'Maya'. Before planting, the field was not treated with nematocides. Pest control and fertilisation were implemented according to integrated pest management (IPM) systems. In the surveyed area, the plant protection products were applied under an approved scheme, authored by Tsolova and Koleva (unpublished data), and complied with the requirements for integrated fruit production (IOBC, IFP). In the scheme, nematicides were not included.

The results in this work were obtained on the basis of field and laboratory investigations. The period of sampling of plant and soil samples was recommended by Knuth *et al.* (2003). Soil and plant root samples were taken randomly at depth 0–25 cm and then transferred in plastic bags to the laboratory.

After mixing the samples, samples of 100 cm^3 were obtained by means of a measuring cylinder. The resulting quantitative and qualitative data was related to this volume. The methods for extraction of the mobile stages of plant-parasitic nematodes from the soil and roots and their subsequent mounting on permanent slides for identification were described previously (Hooper *et al.,* 2005).

A liquid nematode suspension (100 ml) in water was used for extraction of nematodes. The suspension was transferred (5 times per 1 ml) into a Bogorov Modified Counting Chamber. Initially, all nematodes were counted under a stereomicroscope and the plant parasitic nematodes were separated at recount. The average number of live nematodes in the sample (100 cm^3) in the starting suspension of 100 ml was determined as described previously (Peters, 2013). Species characterisation and identification was based on morphology of various life stages (Hooper *et al.,* 1993; Mai *et al*,. 1996; Castillo and Vovlas, 2007; Perry and Moens, 2013; and others). Due to the complexity of the process of identification, some extracted nematodes were determined up to genus level. The dominant genera were described by using Engelmann's method (Engelmann, 1978)

The statistical analysis was carried out with Systat ® 10 software package (SPSS Inc., USA). Analysis of variance (ANOVA) was performed using the General Linear Models procedure and multiple means were compared using Tukey's honest significance difference test at $p < 0.05$.

RESULTS

Before planting strawberry plants, analysis of soil for nematodes indicated low density and migratory endoparasitic nematodes of the genus *Pratylenchus* and ectoparasitic nematodes of the genus *Paratylenchus*. There were no virus vector nematodes of Longidoridae and Trichodoridae, as well as second-stage larva of root-knot nematodes of the genus *Meloidogyne*.

After processing of the soil and plant samples, the nematodes populations were identified and classified to trophic level. Population density of plant parasitic nematodes was higher in soils sampled from strawberry fields in 2013 compared to that in 2014 and 2015. A mean number of 588 ind./100 cm^3 plant-parasitic nematodes were extracted. The largest mean number of plant-parasitic nematodes was observed in 2013 (356.3 ind./100 cm^3), followed by 2014 (135 ind./100 cm^3). The lowest population density of nematodes was in 2015 (96.7 ind./100 cm^3).

The following genera of plant parasitic nematodes were found in soil samples: *Tylenchus*, *Pratylenchus*, *Tylenchorhynchus*, and *Paratylenchus*. Plant parasitic nematodes were not found in plant samples, except in two samples from 2013, where the nematode *Ditylenchus dipsaci* was observed. Leaf nematodes were not found. *Pratylenchus* was the most frequent genus and was found in 91% of 120 soil samples collected in years 2013–2015. *Paratylenchus* spp. were found in 83% of soil samples. Other genera were *Tylenchorhynchus* in 38% and *Tylenchus* in 17% of soil samples. The results of the qualitative analysis showed no significant differences between the species composition of nematode communities extracted from soil and root samples during the research period. Species of the genus *Pratylenchus* were dominant, followed by *Paratylenchus* spp. and *Tylenchorhynchus* sp. Data collected from the three years are presented in Figure 1. *Paratylenchus* species were observed in all years, while *Tylenchus* only in 2014. The most common genera was *Pratylenchus*, which explains why it has been a focus of many studies. Species of this genus are root-endoparasites with great economic importance to this crop. Population density of the species varied significantly between years. Significantly lower density of *Paratylenchus crenatus* and *P. thornei* occurred in 2015 and 2014 than in 2013. Significantly lower population density of *P. neglectus* occurred in 2015 (Table 1).

The density of plant parasitic nematodes (total mean number/100 cm^3 soil) was significantly lower at the end of the study period comparing to the beginning of the study.

The results of the study show that the density of nematodes decreased over the years of cultivation. The influence of the

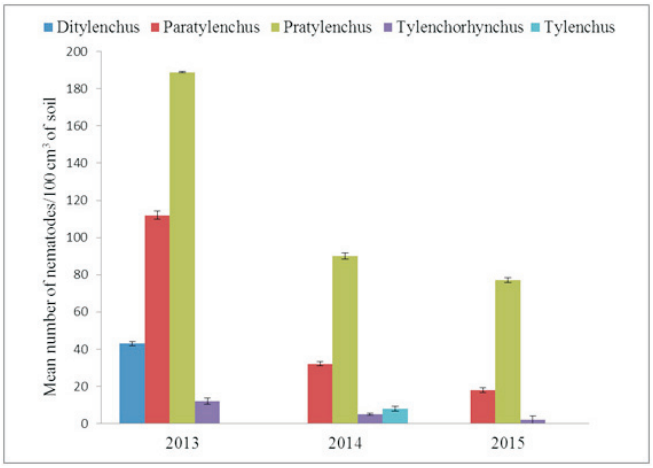

Fig. 1. Mean density of genera of plant-parasitic nematodes extracted from soil sampled from strawberry fields in the sampling period 2013–2015; mean ± SE.

Table 1

MEAN NUMBER OF *PRATYLENCHUS* SPECIES AND TOTAL PLANT PARASITIC NEMATODES EXTRACTED FROM SOIL SAMPLED FROM STRAWBERRY FIELDS IN THE PERIOD 2013–2015

Years	Mean number of nematodes/100 cm^3 of soil			
	P. crenatus	*P. neglectus*	*P. thornei*	Total plant parasitic nematodes
2013	80 ± 0.1a	33 ± 1.3a	76 ± 1.1a	356.3 ± 2.7a
2014	45 ± 1.3b	20 ± 0.9b	27 ± 0.6b	135.0 ± 1.7b
2015	36 ± 0.3b	14 ± 2.1c	25 ± 1.1b	96.7 ± 0.9c

Mean values within a column followed by the same letter are not significantly different using Tukey's honest significance difference test ($p \leq 0.05$); mean ± SE

various factors (application technologies for integrated crop protection) was examined using analysis of variance (ANOVA), which are shown in Table 2. The results indicate sustainable impact of the methods applied for control of the plant parasitic nematode populations.

DISCUSSION

The plant-parasitic nematode genera found in the survey have been reported in surveys of strawberry fields conducted in other regions of Bulgaria (Samaliev and Mohamedova, 2011). New plant-parasitic nematode genera were not found in either roots or soil. *Pratylenchus* was found to be a very common nematode in strawberry fields. The survey confirmed our hypothesis that *Pratylenchus* was the dominant plant parasitic nematode. It was also confirmed that relationships were found between plant parasitic nematode population densities and application of integrated farming practices. *Tylenchus* and *Paratylenchus* were the most common plant parasitic nematodes in the soil of the strawberry fields. *Tylenchus* is not known to cause significant damage to strawberry plants, since most members of this genus are believed to be algal and fungal feeders and not a significant

Table 2

ANALYSIS OF VARIANCE (ANOVA) FOR THE EFFECT OF THE INVESTIGATION PERIOD (APPLICATION TECHNOLOGIES FOR INTEGRATED CROP PROTECTION) ON THE DENSITY OF PLANT PARASITIC NEMATODES EXTRACTED FROM SOIL SAMPLES DURING THE GROWING SEASON OF 2013–2015

ANOVA					
Source of Variation	df	SS	MS	F	Sign. F
Investigation period	1	101140.1667	101140.1667	38.0966	0.0004
Residual	7	18583.833	2654.8333		
Total	8	119724			

threat to plant health (Yeates *et al.*, 1993). *Tylenchorhynchus* are common associates of corn, but are generally considered mild pathogens (Wilson, 2016). In the field trial, nematode numbers were reduced after three years.

Overall, nematode numbers were lower in 2015 than in 2013, which may have been due to the warm dry summer and/or cumulative effects of the integrated plant protection. It is known that the various forms of organic matter and changes in biochemical composition of the soil may suppress populations of plant parasitic nematodes (Akhtar and Malik, 2000; Oka *et al.*, 2000; Stirling, 2014), which is confirmed by our results.

The results showed that integrated plant protection methods can be used to manage damaging plant parasitic nematode populations. Further studies are needed to assess nematode suppression by using various cultivars of this crop.

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AUGIEM PARAZITĀRO NEMATOŽU IEROBEŽOŠANA DĀRZA ZEMEŅU (*FRAGARIA* X *ANANASSA* DUSCHENSE EX ROZIER) INTEGRĒTAJĀ AUDZĒŠANĀ

Aramzemes ir viens no vērtīgākajiem dabas resursiem, un to ilgtspējīga izmantošana ir noteicošais faktors zemeņu integrētajā audzēšanā ilgtermiņā. Ir labi zināms, ka esošās lielogu dārza zemeņu (*Fragaria* × *ananassa* Duchesne ex Rozier) šķirnes ir mazāk izturīgas pret daudzām augiem parazitārajām nematožu sugām un citiem augu patogēniem. Pētījuma mērķis bija izvērtēt audzēšanas tehnoloģiju efektivitāti nematožu kontrolei zemeņu integrētajā audzēšanā. Pētījumi veikti Rietumu Balkānu kalnos Bulgārijā (43°33'22.3"N 22°47'03.4"E), izmantojot šķirni 'Maya'. Pētījumu vietā augu aizsardzības līdzekļi lietoti pēc pieņemtās shēmas saskaņā ar integrētās audzēšanas prasībām. Nematožu populācijas identificētas un klasificētas līdz trofiskuma līmenim. Identificētas šādas nematodes: *Pratylenchus crenatus, P. neglectus*, *P. thornei*, *Tylenchorhynchus* sp. un *Paratylenchus* spp. Parazitāro nematožu blīvums un sugu daudzveidība būtiski samazinājās pētījuma noslēgumā, salīdzinot ar pētījumu sākumu. Pētījuma rezultāti parādīja, ka integrēto audzēšanu var izmantot kā ekonomiski ilgtspējīgu ražošanu augstas kvalitātes ogu iegūšanai, dodot priekšroku videi draudzīgām kaitēkļu ierobežošanas metodēm.