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PLANT-PARASITIC NEMATODES ASSOCIATED WITH STRAWBERRY (*FRAGARIA AIIANASSA* DUCH.) IN BULGARIA

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Abstract

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A survey was conducted in five major strawberry growing regions in Bulgaria (Plovdiv, Blagoevgrad, Ruse, Vratza and Sofia) to study the occurrence of plant-parasitic nematodes associated with strawberry *(*Fragaria ananassa Duch.*)*. A total 205 soil and 109 foliage samples containing mixed populations of 10 genera and 15 species belonging to order *Tylenchida* and *Dorylaimida* were analyzed. The identified species were: *Aphelenchoides fragariae, Aphelenchoides ritzemabosi, Aphelenchoides besseyi, Criconema nutabile, Diylenchus dipsaci, Helicotylenchus dihystera, Longidorus caespiticola, Longidorus elongates, Meloidogyne arenaria, Meloidogyne hapla, Pratylenchus penetrans, Pratylenchus microdorus, Paratylenchus pseudoparietinus, Tylenchorhynchus claytoni and Xiphinema pachtaicum.* Many of the observed species are phytopathologically important parasites of strawberry and some are also vectors of plant viruses. Frequency and density of each species were highly variable from field to field and within the field.

Key words: Plant-parasitic nematodes, strawberry, distribution frequency, population density, Bulgaria

Introduction

Strawberry (*Fragaria ananassa* Duch.) is an important agricultural commodity in Bulgaria. Its cultivation covers 5 800 da with average yield of 907 кg/da (Ministry of Agriculture and Forestry, Bulgaria, 2009). During recent years interest has increased in strawberry cultivation as an alternative cash crop including small family farms in our country. Plant-parasitic nematodes are known to be pests of fruit and vegetable crops (Samaliev and Stoyanov, 2007). In the sixties and seventies, nematode counts in soil and foliage samples indicated that *Aphelenchoides fragariae* (Ritzema Bos) Christie, and some other plant-parasitic nematodes (*Aphelenchoides ritzemabosi* (Schwartz) Steiner, *Aphelenchoides subtensis* (Coobb) Steiner et Buhrer, *Meloidogyne hapla* Chitwood), were spread in strawberry fields in producing regions of Sofia (Stoyanov, 1961, 1975) and Blagoevgrad (Gateva and Budurova, 1975) and at the beginning of 2000 also in Plovdiv region (Samaliev and Stoyanov, 2007). Information on distribution, frequency of occurrence and population density of plant-parasitic nematodes associated with strawberry in Bulgaria is lacking.

Therefore, the objective of this study was to conduct a survey to determine and document the

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occurrence, distribution, density and prevalence of plant-parasitic nematodes in strawberry fields major strawberry producing regions in Bulgaria.

Material and Method

A survey was conducted in different locations in the major strawberry growing regions of Plovdiv, Blagoevgrad, Ruse, Vratza and Sofia. Sampling was conducted during April-June 2008-2009. Soil, root, and foliage (leaves, stem and flower-buds) samples were collected randomly from the most fields of each region (Figure 1). Each soil sample was composite of 10-15 soil cores from the same field, collected randomly to a depth of 20-cm with a hand shovel. Samples were placed in plastic bags, sealed and brought back to the laboratory and stored at 5°C until processed for nematode extraction and were examined in a Laboratory of Nematology at Agricultural University, Plovdiv. Root-knot nematodes were extracted from roots which were washed free of soil and examined for galling and root-knot infection. Root-knot nematodes were isolated from galled roots and identified by the examination of perinea patterns of adult females as well as the characters of the second-stage juveniles (Eisenback et al., 1981).

Fig. 1. Map of Bulgaria; showing sampling sites in the major of strawberry production region, Bulgaria

Vermiform nematodes were recovered from two 100 cm^3 sub samples (after thorough mixing of the soil from each sample) by Cobb's sieving and decanting technique followed by a modified Baermann funnel method (Hooper, 1986). An incubation method was used to extract the nematodes from strawberry roots (Young, 1954). Strawberry foliage samples were randomly chosen from each region. Within 24 h of arrival at laboratory, all the stems, buds and leaves in each sample were cut into small pieces (0.5-1 cm) placed in bags and mixed. A two 15-g sub sample from each sample was used for nematode extraction using Baermann funnel for 3 days. Isolated nematodes were killed at 65°C and fixed in 4% formalin and placed in viols. Prior to counting, solution containing nematodes were agitated thoroughly and 3 ml poured to a counting dish. Nematodes were counted using a stereo binocular microscope. Counting of root-knot nematodes based on second stage juveniles (J_{2S}) only. Selected specimens for each of the recorded species were processed for dehydration by Seinhorst's (1959) rapid glycerin method and mounted on plastic slides in anhydrous glycerin. Identification of species of plant parasitic nematodes was based on the morphology and measurements of adults (Perry et al., 1959; Esser, 1973; Handoo and Golden, 1989; Handoo, 2000; Siddiqi, 2000).

Simple statistics (frequency and density) were performed on nematode counts using SPSS-12 program.

Results

Nematodes in 10 genera and 15 species belonging to order *Tylenchida* (Filipjev) Thorne and *Dorylaimida* Pearce, were found in association with strawberry (*Fragaria ananassa* Duch.) from total 205 soil and 109 and foliage samples (Table 1 and Table 2).

Table 1
Occurrence frequency and density of plant-parasitic nematodes in strawberry fields (in 100 cm^3 soil) \ in major strawberry growing regions, Bulgaria

Nematode species	Strawberry growing regions / number of samples collected									
	Plovdiv / 35		Blagoevgrad / 30		Vratzca / 40		Ruse / 70		Sofia / 30	
	RF*	MD**	RF	MD	RF	MD	RF	MD	RF	MD
Aphelenchoides fragariae	-	-	-	-	5	7 ±1.7	-	-	-	-
Aphelenchoides ritzemabosi	-	-	-	-	7.5	9 ±2.2	-	-	-	-
Aphelenchoides besseyi	2.8	5 ±1.7	-	-	-	-	-	-	-	-
Criconema nutabile	-	-	-	-	12.5	10 ±14.6	17.1	32 ±19.5	3.3	41 ±33.6
Ditylenchus dipsaci	11.4	8 ±4.1	13.3	9 ±3.4	-		15.7	16 ±5.7	10	13 ±4.2
Helicotylenchus dihystera	-	-	11.1	35±16.9	30	21 ±7.8	21.4	18 ±5.6	33.3	29 ±10.5
Longidorus caespiticola	5.7	10 ±3.6	-	-	5	6±4.6	8.6	5±2.6	-	-
Longidorus elongatus	2.8	5 ±1.1	-	-	7.5	9 ±3.5	-	-	-	-
Meloidogyne arenaria	31	61 ±22.1	26.6	49 ±17.4	22.5	19 ±13.1	20	14 ±10.4	20	16 ±13.1
Meloidogyne hapla	26	55 ±35.4	36.6	70 ±47.8	42.5	111±48.3	41.4	125 ±56.0	30	89 ±55.4
Pratylenchus penetrans	37	28 ±16.1	33.3	19 ±10.4	26	15 ±13.1	45.7	28 ±15.7	20	21 ±10.2
Pratylenchus. microdorus	-	-	-	-	12.5	17±9.6	15.7	25 ±13.5	-	-
Paratylenchus pseudoparietinus	-	-	-	-	10	76±24.6	-	-	13.3	65 ±25.5
Tylenchorhynchus claytoni	-	-	10	21±8.4	5	31 ±11.8	7.1	21 ±7.8	-	-
Xiphinema pachtaicum	8.6	10±3.8	6.6	16 ±3.8	-	-	10	22 ±6.8	-	-

*RF = Relative frequency of occurrence (percentage of samples in which species was found); **MD = Mean density ± standard deviation of nematodes in 100 cm^3 soil.

Plant-parasitic nematodes on soil samples

Root-knot northern *Meloidogyne hapla* Chitwood and peanut *M. arenaria* (Neal) Chitwood nematodes, associated with strawberry, were the

Table 2

Occurrence frequency and population density of *Aphelenchoides fragariae*, *Aphelenchoides ritzemabosi*, *Aphelenchoides besseyi* and *Ditylenchus dipsaci* in strawberry plant tissues

Nematode species	Strawberry growing regions / number of samples collected				
	Plovdiv / 17	Blagoevgrad /15	Vratza / 20	Ruse / 42	Sofia / 15
Aphelenchoides fragariae					
Occurrence frequency*	35.3	26.6	60	64.2	53.3
Nematodes densily**	34 ±7.5	42 ±14.5	70±20.1	98 ±43.8	61 ±10.6
Aphelenchoides ritzemabosi					
Occurrence frequency*	41.1	53.3	25	28.6	26.6
Nematodes densily**	70 ±15.6	66 ±18.5	23±9.4	29 ±13.8	31 ±12.6
Aphelenchoides besseyi					
Occurrence frequency*	11.7	-	-	7.1	6.7
Nematodes densily**	8 ±1.6	-	-	11 ±2.8	9.0 ±1.8
Ditylenchus dipsaci					
Occurrence frequency*	11.7	13.3	10	14.3	20
Nematodes densily**	14 ±5.6	8 ±2.5	13±4.4	16 ±5.8	11 ±3.6

* Percentage of samples in which nematode was found; **Mean ± standard deviation of nematodes per 15 g fresh tissue of strawberry plant.

most conspicuous plant-parasitic nematodes of this group, collected from all surveyed regions. However, their occurrence frequency and density were highly variable, as the highest frequency and density for *M. hapla* was recorded in Vratza (42.5% and 111 nematodes/100 cm^3 soil) and Ruse (41.4% and 125 nematodes/100 cm^3 soil), and for *M. arenaria* was in Plovdiv (31% and 61 nematodes/100 cm^3 soil) and Blagoevgrad (26.6% and 49 nematodes/100 cm^3 soil), Table 1. The lowest frequency and density for *M. hapla* was in Plovdiv (26% and 55 nematodes/100 cm^3 soil) and Sofia (30% and 89 nematodes/100 cm^3 soil), and for *M. arenaria* – Ruse (20% and 14 nematodes/100 cm^3 soil) and Sofia (20% and 16 nematodes/100 cm^3 soil), Table 1. Of the soil samples infested with *Meloidogyne* spp., 48.0% had *M. hapla,* 27% had *M. arenaria* and 25% had both species.

The other proven parasite of strawberry found in this study was root-lesion nematodes *Pratylenchus penetrans* Cobb and *P. microdorus* Andrassy. *Pratylenchus penetrans* was the predominat species and was found of the samples at the all regions (Plovdiv, Blagoevgrad, Vratza, Ruse and Sofia) and were present in 37, 33.3, 26, 47.5 and 20% of sampled fields with density 28, 19, 15, 28 and 21 nematodes/100 cm^3 soils, respectively. The other species *P. microdorus* Andrassy was collected only from Vratza and Ruse regions in 12.5 and 15.7% of sampled fields (density 17 and 25 nematodes/100 cm^3 soil) , respectively (Table 1) in mix population with *P. penetrans*.

The pin nematode *Paratylenchus pseudopariet-inus* (Micoletzky) Micoletzky, was plant-parasitic nematode with the highest density in Vratza and Sofia in tested strawberry fields. No information is presently available on the pathogenicity of this ectoparasitic genus on strawberry plants.

Six other plant-parasitic nematode species on roots, *Criconema nutabile* (Taylor) Raski - Luc*, Helicotylenchus dihystera* (Cobb) Sher*,*

Longidorus elongatus (de Man) Thorne et Swanger, *L. caespiticola* Hooper, *Tylenchorhynchus claytoni* Steiner and *Xiphinema pachtaicum* (Tulaganov) Kirjanova, were also found occasionally and in low density (Table 1).

Plant-parasitic nematodes on foliage (leaves, stem and flower-buds)

Strawberry bud *Aphelenchoides fragariae* (Ritzema Bos) Christie and chrysanthemum *Aphelenchoides ritzemabosi* (Schwartz) Steiner nematodes, were predominant species from above-ground plant parts collected from all surveyed regions (Table 2). Occurrence frequency and density of both species were highly variable in different investigated regions. For instance, the highest frequency and density of *Aph. fragariae* was recorded in Ruse (64.2% and 98 nematodes/15 g fresh tissue), Vratza (60% and 70 nematodes/15 g fresh tissue) and Sofia (53.3% and 61 nematodes/15 g fresh tissue) and the lowest was in Blagoevgrad (26.6% and 42 nematodes/15 g fresh tissue) and Plovdiv (35.3% and 34 nematodes/15 g fresh tissue) (Table 2). Vice versa for *Aph. ritzemabosi,* the highest frequency and density was recorded in Blagoevgrad and Plovdiv (53.3% and 41.1% and 70 and 66 nematodes/15 g fresh tissue) and the lowest – Ruse, Vratca and Sofia (Table 2). Of the foliage samples infested with *Aphelenchoides* spp., 22% had *Aph. fragariae*, 12% had *Aph. ritzemabosi*, and 66% had both species.

Two other plant-parasitic nematode species on foliage *Aphelenchoides besseyi* Christi and stem nematode *Ditylenchus dipsaci* (Kfflin) Filipjev, were also found occasionally.

Discussion

This survey provides information on occurrence and density of plant-parasitic nematodes associated with strawberry crops in the major strawberry growing regions in Bulgaria. Some nematodes identified in this survey are of economic importance and are considered serious pests of strawberry species and were found in the samples of all strawberry growing regions investigated - Plovdiv, Blagoevgrad, Ruse, Vratza and Sofia. These nematodes were *Aph. fragariae, Aph. ritzemabosi, M. hapla, M. arenaria and P. penetrans*. *Aphelenchoides fragariae* and *Aph. ritzemabosi* are also known as foliar nematodes are parasites of above-ground plant part and may be endo- or ectoparasitic, produce small curled or crinkled leaves (crimp), deformed buds and flowers. *Aphelenchoides fragariae* and *Aph. ritzemabosi* were found in soils from Vratca only. They might have disseminated through rain water, the infected buds or leaves which may have fallen a few days before sampling. In most of samples both species are in mixed population. This widespread distribution of *Aphelenchoides* spp. in all major strawberry regions is consistent with results from earlier surveys of strawberry crops in Sofia and Blagoevgrad region (Stoyanov, 1975; Gateva and Budurova, 1975). *Meloidogyne hapla* and *M. arenaria* were found in the soil or as sedentary endoparasites in roots and induce root galls. Earlier surveys (Gateva and Baycheva, 1976; Stoyanov, 1980; Samaliev and Stoyanov, 2007) also showed a widespread distribution of *M. hapla* and *M. arenaria* on tobacco in Southwestern and Central Bulgaria and *M. hapla* localized in single fields on strawberry in Blagoevgrad (Gateva and Budurova, 1975) and Plovdiv (Samaliev and Stoyanov, 2007) regions. Knowledge, of which species are present in a field, will enable the grower to implement the proper rotation sequence in addition to other control practices, thereby suppressing root-knot nematode populations to increase strawberry fruit quality. *Pratylenchus penetrans* is a migratory endoparasite of roots, and its infection makes fine reddish-brown lesions on rootlets. It is also known to enhance the severity of *Gnomonia comari* (fungus) (Kurppa and Vrain, 1989) and *Rhizoctonia fragariae* (LaMondia, 2003) on strawberry roots. It is reported that *P. penetrans* and *Aph. fragariae* have been most frequently associated with strawberry damage in Blagoevgrad region (Gateva and

Budurova, 1975).

Presence of *Aph. fragariae, Aph. ritzemabosi, M. hapla, M. arenaria and P. penetrans* nematodes in strawberry fields indicates a risk of damage, and recommended management strategies should be taken by growers to prevent them from increasing and spreading.

Strawberries, in this investigation, are also host of *D. dipsaci, Logidorus* sp. and *Xiphinema* sp. All of these nematodes are potential pathogens to strawberry and their identification in strawberry plantings or in land to be planted to strawberries should be cause of concern (Samaliev and Stoyanov, 2007).

Other nematode species identified in this survey are ectoparasites of roots and have not been documented as dangerous pests of strawberry to date. They include *H. dihystera and T. claytoni.* The species of *Criconemoides, Helicotylenchus, Tylenchorhynchus* and *Longidorus* have also been commonly found in samples from virus vectors they can be damaging at very low population levels.

Conclusion

The results of this survey indicate that plant-parasitic nematodes on strawberry fields are widely distributed in Bulgaria. Several of the recorded species are known pathogens of strawberry and are capable of causing damage to the crop. Knowledge of which species and race is present in a field is important to determine the possible threat to strawberry and its control. The damage thresholds are also needed to be established in the soil types and under the growing conditions of different regions of Bulgaria. Particularly the population dynamics of *Aphelenchoides* spp.*, Meloidogyne* spp. and *Pratylenchus* spp. on strawberry and their interactions with other soil bourn disease organisms deserve further studies. Our results provide essential background information needed for planning and practicing nematode management strategies in Bulgarian strawberry fields.

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