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POPULATION DYNAMICS OF *Longidorus attenuatus* (Nematoda: Longodoridae) AND *Tylenchorhynchus claytoni* (Nematoda: Dolichodoridae) ON RED RASPBERRY AND BLACK CHOKEBERRY

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Abstract

Plant parasitic nematodes Longidorus attenuatus Hooper and Tylenchorhynhus claytoni Steiner are serious pests on both red raspberry Rubus idaeus L. and black chokeberry Aronia melanocarpa (Michx.) Elliott grown in Bulgaria. Considering their importance as root parasites, the present study is a first step of evaluations done on nematode densities and seasonal fluctuation of their populations. Throughout one year survey, the highest population density of L. attenuatus was found at 20 cm soil depth, while the T. claytoni population was observed highest at 10 cm soil depth. The peak of the populations of the phytonematodes was observed in June and July on red raspberry and black chokeberry, respectively. The highest number of 321 individuals/100 cm^3 soil of nematodes was found in raspberry field, while the number of 223 individuals/100 cm^3 soil was observed in chokeberry plantation. The obtained data of population dynamics of L. attenuatus and T. claytoni clearly indicates the most appropriate time for control measures applying. The symptoms observed on root systems demonstrate the negative impact of nematode feeding and development on them. Further evolutions should be focused on searching of alternative methods of control of plant parasitic nematodes on red raspberry and black chokeberry.

Key words*: plant parasitic nematodes, population density, soil depth, Rubus ideus, Aronia melanocarpa, root damage.*

INTRODUCTION

In last decade, production of small fruit crops has significantly increased in Bulgaria. Red raspberry *Rubus idaeus* L. is a traditional agricultural crop that has been grown both in conventional and organic fields. During 2014-2017, the total area of raspberry plantation has increased by 34% (Agrostatistica, 2018) and approximately 2/3 of fruit production has been exported to western European countries and Asia. Black chokeberry *Aronia melanocarpa* (Michx.) Elliott has attracted the interest of the growers due to both considerable high price of the fresh and frozen fruits and plants resistance to drought and low temperature.

In many cases, the import of uncertified raspberry and chokeberry plant materials is the main reason for entrance and spread in the territory of the country of numerous pest and pathogens, including plant parasitic nematodes. Also, the change of cultivar assortment leads to growing of poor adapted to the climatic and soil conditions plants which are severe attacked by soil borne pathogens and nematodes. As a consequence, the productive lifetime of berry plantings in several regions of the country has drastically decreased up to 5-7 years.

Approximately 4,100 species of phytonematodes have been identified worldwide (Decramer and Hunt, 2006), new species are continually being described while others, previously considered as non-damaging on certain crops are becoming pests on them as planting patterns change. Although plant parasitic nematodes are viewed as one of the most insidious and costly pest group on berry plants (Bélair, 1991), data on their reproduction, population dynamic, feeding, species composition and economic impact remain less than comprehensive, especially for black chokeberry.

In berry plants, phytonematodes are able to cause direct damages on their root systems (Bélair and Khanizadesh, 1994), as well as to transmit plant nepovirusses (Taylor and Brown, 1997). Species of genus *Tylenchorhynchus* are known as typical ectoparasites, destroying the root tissues and lead to stunt growth (Poiras et al., 2014). *Longidorus* spp. feed on roots, causing necrosis and small gall formations, but spe-

cies *Longidorus elongatus* and *Longidorus macrosoma* transmit the Raspberry ringspot nepovirus (RRSV) (Taylor and Brown, 1997). *L. elongatus* and *L. macrosoma* have been reported several times as pests on red raspberry in Bulgaria (Stoyanov, 1980; Choleva et al., 1992; Samaliev and Stoyanov, 2008; Tzolova and Koleva, 2015). At the same time, there is an absence of information concerning the species *Longidorus attenuatus* Hooper and *Tylenchorhynhus claytoni* Steiner on red raspberry. In addition, data on occurrence and population density of plant parasitic nematodes associated with black chokeberry in Bulgaria is lacking.

The aim of the present study was to provide preliminary information on seasonal fluctuation of two of the most important nematode species *L. attenuatus* and *T. claytoni* associated with *R. ideus* and *A. melanocarpa* throughout one year at three soil depths.

MATERIALS AND METHODS

Three-years-old raspberry plantation (cv. Lyulin, 2.2 ha) and four-years-old black chokeberry plantation (cv. Viking, 1.8 ha), both with a history of phytonematode infestation were selected for this survey.

The commercial plantations are located (N 41.51 E 24.74 - raspberry, and N 41.53 E 24.76 - black chokeberry) near Smilyan village, South Bulgaria. The soil type is sandy-loam. Ten sites of each field (raspberry and black chokeberry) with well-defined spots of chlorotic and wilted plants were selected and marked as permanent sampling sites.

To determine population dynamics of *Longidorus attenuatus* and *Tylenchorhynchus claytoni*, soil samples were collected from the sampling sites by using a soil auger at monthly intervals for a period of one year from January to December, 2017. Each composite soil sample was obtained by randomly collected five soil cores within sampling sites, in depth of 10 cm, 20 cm and 40 cm.

Sampling position were 15-25 cm apart from the plant stem. The samples were placed in plastic bags, labeled and transported to Laboratory of Entomology end Nematology at Agricultural University, Plovdiv where the samples were stored at 7-10°C until processed for nematode extraction.

To extract the nematodes from the soil, four 100 cm^3 sub-samples were processed using Cobb's sieving and decanting technique followed by a modified Baerman funnel method (Hooper, 1986). Extracted nematodes were killed in 65 °C hot water, fixed in TAF and placed in labeled containers (Bezoojen, 2006). Nematodes were counted under dissecting stereomicroscope. Selected specimens were processed to dehydratation followed Seinhorst's (1959) rapid glycerine method and mounted on permanent slides. To identify and confirm nematode species, the taxonomic keys were used (Romanenko, 1978; Loof and Chen, 1999; Handoo, 2000, Siddiqui, 2000). The obtained data were statistically analyzed for critical difference (CD) at $p = 0.05$ and $p = 0.01$ (Panse and Sukhatme, 1978).

RESULTS AND DISCUSSIONS

Seasonal changes in population density of *Longidorus attenuatus* and *Tylenchorhynchus claytoni* at different soil depths (10 cm, 20 cm and 40 cm) in the rizosphere of red raspberry and black chokeberry plants in 2017 are presented in Table 1 and Table 2. The results indicate that the number of plant parasites at different soil layers is influenced by the seasonal climatic condition.

During the spring, summer and autumn months, the populations of *L. attenuatus* and *T. claytoni* were observed higher than winter months in both raspberry and chokeberry fields. However, the highest density of *L. attenuatus* was found in the middle (20 cm) layer and the number of nematodes on raspberries varied from 4 individuals/100 cm^3 soil in September to 59 individuals/100 cm^3 soil in June (Table 1). On chokeberries, the number of *L. attenuatus* varied from 11 individuals/100 cm^3 soil in March to 52 individuals/100 cm^3 soil in July (Table 2). Throughout the same three seasons, *T. claytoni* showed different pattern of movement into the soil depths and the highest number of the parasites was observed in the upper (10 cm) layer. The maximum in population density of *T. claytoni* was found in June (176 individuals/100 cm^3) on *R. ideus* and in July (95 individuals/100 cm^3) on *A. melanocarpa*, while the minimum in population density was observed in March for both raspberry and

chokeberry fields (19 and 9 individuals/100 cm^3, respectively) (Tables 1 and 2). During the winter season, both species *L. attenuatus* and *T. claytoni* moved deeper into the soil and their highest population densities were observed in the lower (40 cm) layer. The highest number of the parasites was found in January. On *R. ideus*, 20 and 6 individuals/100 cm^3 soil of *L. attenuatus* and *T. claytoni* were counted, respectively, while on *A. melanocarpa* the number of nematodes was 7 and 11 individuals/100 cm^3 soil, respectively.

Table 1. Population densities of two plant parasitic nematodes *Longidorus attenuatus* and *Tylenchorhynchus claytoni* associated with red raspberry cv. Lyulin throughout one year at different soil depth

Months/Season	*Longidorus attenuatus*			Total	*Tylenchorhynchus claytoni*			Total
	10 cm*	20 cm*	50 cm*		10 cm*	20 cm*	40 cm*	
January (Winter)	5	7	20	32	1	43	6	50
February (Winter)	8	10	14	32	7	49	6	62
March (Spring)	11	20	17	48	19	65	5	89
April (Spring)	11	29	18	58	38	81	2	111
May (Spring)	33	41	9	83	129	52	0	181
June (Summer)	43	59	2	104	176	41	0	217
July (Summer)	14	32	25	71	95	27	0	122
August (Summer)	2	9	21	32	36	50	4	90
September (Autumn)	6	4	29	39	26	40	6	72
October (Autumn)	16	34	6	56	67	37	8	112
November (Autumn)	10	21	10	41	24	46	10	80
December (Winter)	8	13	18	38	16	39	4	59
CD (p = 0.05)	10.02	11.16	9.18	16.32	9.80	10.65	5.01	15.22
CD (p = 0.01)	14.34	13.98	12.56	19.44	12.76	14.03	8.28	18.64

*Data represents the mean of four replicates.

Table 2. Population densities of two plant parasitic nematodes *Longidorus attenuatus* and *Tylenchorhynchus claytoni* associated with black chokeberry cv. Viking throughout one year at different soil depth

Months/Season	*Longidorus attenuatus*			Total	*Tylenchorhynchus claytoni*			Total
	10 cm*	20 cm*	40 cm*		10 cm*	20 cm*	40 cm*	
January (Winter)	1	4	7	12	0	24	11	35
February (Winter)	10	9	7	26	4	36	13	53
March (Spring)	11	11	10	32	9	39	5	53
April (Spring)	16	15	5	36	19	50	3	72
May (Spring)	21	27	3	51	25	65	0	90
June (Summer)	27	33	1	61	71	57	0	128
July (Summer)	26	52	4	82	95	51	0	141
August (Summer)	7	15	8	30	42	34	4	80
September (Autumn)	9	13	6	29	46	30	8	84
October (Autumn)	17	24	5	46	40	42	14	96
November (Autumn)	9	14	8	31	20	22	11	53
December (Winter)	5	9	11	25	8	17	10	35
CD (p = 0.05)	8.45	10.12	8.09	11.57	14.22	12.66	9.15	15.24
CD (p = 0.01)	12.03	14.06	11.35	15.09	17.53	15.98	13.01	18.85

*Data represents the mean of four replicates.

Zuckerman and Strich-Harari (1963) stated that under a temperate climate the population densities of plant parasitic nematodes on perennial plant species peaked in summer season, while the lowest population densities occurred in winter season. Jones (1980) added that the temperature is the major environmental factor limiting nematode population density in temperate zone of the world. According to Badra and Caveness (1983) the rainfalls and soil moisture should be considered as the most important factors influenced the nematode populations under a subtropic and tropic condition of the world. The results in this study showed the population of *L. attenuatus* and *T. claytoni* reached their highest densities during May-July, and their lowest densities during December-February (Tables 1 and 2). Similarly, Afreh-Nuamah and Hemeng (1995) found that the populations of *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* on plantain increased in June-July and decreased in December-January. Contrariwise, Mani and Al-Hinai (1996) reported that the highest densities of *Helicotylenchus multicinctus*, *Meloidogyne incognita* and *Tylenchorhynchus coffeae* associated with banana plants occurred in February-April, and their lowest densities observed in May-July.

During the active growing season of *R. ideus* and *A. melanocarpa,* population of *L. attenuatus* was concentrated in the middle soil layer, especially in the spring and summer months (Tables 1 and 2). Unlike, the highest population density of *T. claytoni* on red raspberry and black chokeberry occurred in the upper soil depth, notably in the summer months (Tables 1 and 2). Likewise, Siddiqui (2005) reported the highest population densities *of Hoplolaimus indicus*, *Helicotylenchus indicus*, *Xiphinema americanum*, *Pratylenchus coffeae*, *Tylenchulus semipenetrans* and *Hemicriconemoides mangiferae* on orange *Citrus reticulata* and lemon *Citrus aurantifolia* at 10 cm soil depth. In addition, Siddiqui (2007) observed the highest number of 213-360 individuals/200 g soil of *Tylenchorhynchus mashoodi* on mango *Mangifera indica* at 10 cm soil depth during July-September.

Fluctuation of phytonematode populations on red raspberry and black chokeberry fields is presented on Figure 1. Population densities of both *L. attenuatus* and *T. claytoni* had one pronounced peak, in June (321 individuals/100 cm^3) and July (223 individuals/100 cm^3), respectively on raspberry and chokeberry. A second increase but lower peak was observed in October, when the total number of nematodes reached 158 individuals/100 cm^3 and 142 individuals/100 cm^3, respectively on *R. ideus* and *A. melanocarpa* (Figure 1).

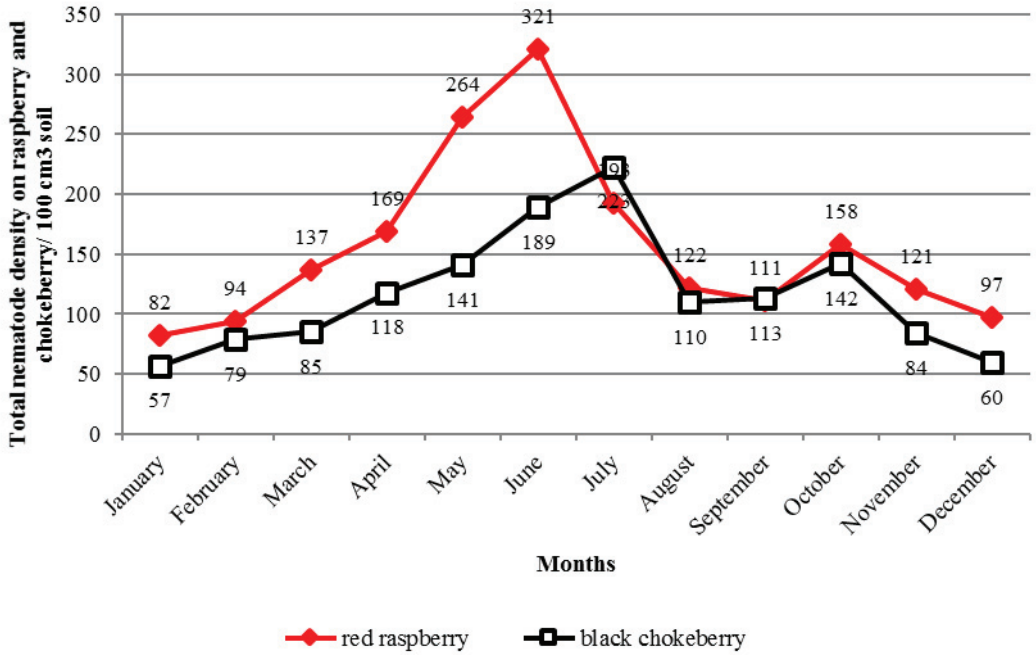

Figure 1. Fluctuation of population densities of plant parasitic nematodes on *Rubis ideus* and *Aronia melanocarpa* in 2017

On other hand, the lowest nematode density was found in January. In this month the total number of the parasites was 82 individuals/100 cm^3 and 57 individuals/100 cm^3, respectively on raspberry and chokeberry. During March-June populations of *L. attenuatus* and *T. claytoni* increased, but in August and September distinctly decreased (Figure 1). The low soil temperature (the data is not presented) in December-February might be suspected to be responsible for the reduction in the plant parasitic nematode populations. Moreover, the quiescence of berries growth during the winter months could be also related to the nematode population decline. Reduction in the total number of nematodes in August-September is probably due to increase in soil temperatures. In drought conditions the parasites might be stay in anabiosis or diapause or migrate deep (up to 70 cm) into the soil (Oostenbrink, 1966). Similarly to our findings, Kella et al. (2011) reported that the population of *Meloidogyne incognita* associated with banana *Musa* spp. increased during June-August and then decreased during November-January.

Contrariwise, Eissa et al. (2013) observed two consecutive peaks (in January and in February) and one lowest level (in October) in population

density of *Helicotylenchus exallus* associated with banana plants.

Figure 2. Dying root system of red raspberry due to feeding of *Longidorus attenuatus* and *Tylenchorhynchus claytoni*

The presence of *L. attenuatus* and *T. claytoni* on both red raspberry and chokeberry fields and their feeding on plant roots lead to well distinguishable symptoms of wilting and swelling of the root systems (Figure 2).

Constantly, the question raised by the growers is how to assume nematode damages on the berry plants? Some of typical symptoms on nematode feed on roots were detected of plants had showed the tendency of yellowing and wilting of raspberry and chokeberry leaves and shoots.

When were uprooted these plants had a chlorotic and necrotic stubby roots, lack of lateral root development and reduction of root weight (Figures 3 and 4).

As a consequence, plants become unthrifty and died from inability to take up sufficient water and nutrients.

Figure 3. Absence of lateral roots, necrosis and small galls of root tips of red raspberry plants infested with nematodes

Figure 4. Swelling, yellowing and malformed roots of black chokeberry attacked by nematodes

CONCLUSIONS

From the results above can be concluded that the populations of *Longidorus attenuatus* and *Tylenchorhynchus claytoni* are concentrated up to 20 cm soil depths during the active growing season of *Rubus ideus* and *Aronia melanocarpa*. This should be considered when applying nematicides or other products for plant protection or for plant growth promotion. Population densities of *L. attenuatus* and *T. claytoni* are highest in May-July, when is the most appropriate time for nematode treatment. Another increase in nematode population is observed in October. It is important to note, that November under the climatic condition in

Bulgaria is a typical autumn month and when is necessary the nematicides could be also applied in this period.

Under favorable condition and susceptible cultivars, symptoms of necrosis and swelling of root systems are observed. Infested and damaged roots lead to wilting and yellowing of the plants and overall to plant dead.

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