MONITORING OF POTATO CYST NEMATODES (*GLOBODERA* SPP.) IN SOUTHWEST BULGARIA

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Abstract
The potato cyst nematodes, *Globodera pallida* and *Globodera rostochiensis*, can cause significant economic damage to potato production and their conduct is a difficult process. They are quarantine pests for Europe, which are regulated by different directives specific to different regions. The main objective of the study was to provide data of the determination of the spread of potato cyst nematodes in Southwest Bulgaria in order to limit their further dissemination outside the infected areas. In the period 2015-2016, the monitoring of potato cyst nematodes was carried out in six major potato-producing regions in Bulgaria (Samokov, Pernik, Kyustendil, Pazardzhik, Blagoevgrad and Smolyan). The monitoring has covered seed production areas and potato areas intended for human consumption and/or for processing. The results confirm the presence of both species in the observed potato-growing areas. The distribution and density of nematodes of the genus *Globodera* varies between regions. *G. rostochiensis* was the most common species in all areas. The species was found by itself in a higher level than in joint populations of *G. pallida*.

Key words: *Globodera pallida*, *G. rostochiensis*, distribution.

Introduction

Potato (*Solanum tuberosum*) is one of the most important food sources in the world (FAO 2008, Lutaladio and Castaldi 2009). The potato production in Bulgaria is concentrated in four main regions: Pazardjik, Plovdiv, Smolyan and Samokov. Significant economic damage to potato production worldwide can cause the plant parasitic nematodes. The estimation of potato yield showed that the losses can reach up to 9% per year as a result of attack by the potato cyst nematodes of the genus *Globodera* (Olsson, 2009). Many different control strategies have been developed to reduce the damage effect caused on potato crops, as a chemical control (Norshie et al. 2016), crop rotation (Evans and Haydock 2000, Eberlein et al. 2016) and use of resistant cultivars of potatoes (Sparkes, 2016). Potato cyst nematodes *Globodera pallida* (white potato cyst nematode) and *Globodera rostochiensis* (yellow potato cyst nematode) are quarantine pests and are regulated by various plant protection directives specific to the different regions. In Europe the spread and control of potato cyst nematodes is regulated by COUNCIL DIRECTIVE 2007/33/EC of 11 June 2007. The main ways of preventing and limiting the spread of potato cyst nematodes are the areas intended for seed propagation and the plants or plant parts intended for planting to be free of these nematodes. The current situation in the EU requires extensive studies on the spread and density of potato cyst nematodes and to develop new strategies to control them. The main objective of the study was to provide data of the determination of the spread of potato cyst nematodes in Southwest Bulgaria, in order to limit their further dissemination outside the infected areas.

Material and methods

The results of this work were obtained on the basis of field observations and laboratory analysis from March 2015 to October 2016. Six observation points were selected (Pernik, Kyustendil, Samokov, Pazardzhik, Blagoevgrad and Smolyan), on which potatoes were grown. The studies on the

presence and spread of potato cyst nematodes *Globodera pallida* (Stone, 1973), *Globodera rostochiensis* (Wollenweber, 1923) (Heteroderidae) included potato-growing areas intended for planting and for consumption and processing.

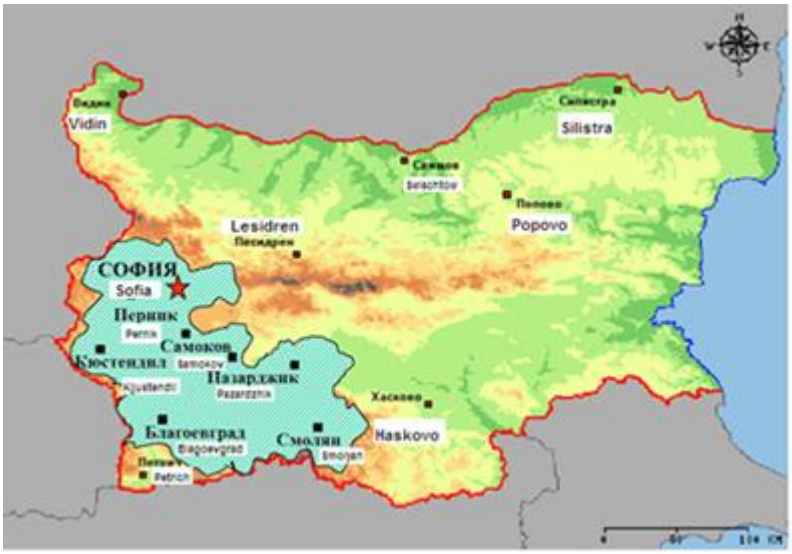

Figure 1. Regions in Southwestern Bulgaria, subject to monitoring of potato cyst nematodes

The soil samples were taken according to the procedure under Ordinance 17 of 3 June 2010 on the control of potato cyst nematodes (Ministry of Agriculture, Food and Forestry of Republic Bulgaria) during harvesting and before planting the potatoes. After mixing of the samples taken, average samples of 100 cm3 were compiled using a measuring cylinder. The resulting quantitative and qualitative data refer to this volume. The samples were placed in plastic bags, sealed and stored in a refrigerator at 4-5 °C to carry out laboratory analysis. Various extraction methods were used for the isolation and quantification of the cysts according to established methodology. The methods for the extraction of the life stages of the potato cyst nematodes (second-stage juveniles J2 and male) from the soil are according to the Cobb and Baermann technic. The vitality of eggs and juvenile stages was determined by macroscopic and microscopic analysis and by an egg-hatch test. The cysts, live juvenile stages and males in soil samples and live juvenile stages and cysts eggs were quantified. The average number of live nematodes in the sample (100 cm3) at the starting suspension of 100 ml was determined by Peters (2013). Species characterization and identification were based on morphology of various life stages ad cysts (Subbotin et al. 2010, EPPO 2013). In addition, the samples were examined for the presence of cyst nematodes by flotation methods.

Results and discussion

Monitoring of areas for the production of potatoes for consumption and processing

In 2016, the surveys were carried out in potato fields amounts to 18.400 ha, which is 15% of the total harvested areas in 2015 (122.484 ha). In 2015-2016, the production of 597 registered and 71 unregistered producers of potatoes was controlled.The plant-health control of potatoes has been carried out in the potato-growing areas of 30.1528 ha, but only of potato cyst nematodes in 9.8 ha areas; 660 soil samples were analysed. The identified plant-parasitic nematodes belong to the order Tylenchida and order Dorylaimida. The cyst nematodes were identified as: *Globodera rostochiensis* (Wollenweber 1923) *Globodera pallida* (Stone 1973) and *Heterodera* spp. All *Globodera* cysts, isolated from the six regions of Southwestern Bulgaria were morphologically identified to species level as *G. rostochiensis* and *G. palida*, located in the following areas:

- *G. pallida* - in 50 soil samples (5 Smolyan, 9 Sofia-District, 34 Samokov, 2 Pazardzhik);
- *G. pallida* /dead stages/ - in 1 soil samples (1 Pazardzhik);
- *G. rostochiensis* - in 63 soil samples (42 Sofia-District, 20 Samokov, 1 Pazardzhik);

- *G. pallida* + *Globodera rostochiensis* - in 1 soil sample (Sofia-district).

Table 1. Distribution of established species *G. pallida* and *G. rostochiensis* in the observed areas

	G. pallida	*G. rostochiensis*	*G. pallida + G. rostochiensis*	Total number of infected samples
Blagoevgrad				
Pazardzhik	2+1 /dead stages/	1		3
Smolyan	5	20		25
Sofia district	9	42	1	52
Samokov	34			34
Pernik	0			
Kyustendil	0			
Total:	50	63	1	113

As a result of the control, in the year 2015-2016, a total of 1.6 ha (124 areas) were mapped:

Monitoring of potato seed production areas

In the soil samples from the surveyed areas before planting (1.9 ha) and in those, taken during the vegetation period from the seed production areas, the nematodes of genus *Globodera* were not found. The obtained results overlap with the studies conducted by Samaliev and Stoyanov (2007) and Samaliev (2011) and confirm the prevalence of the two species of potato cyst nematodes in Southwestern Bulgaria. Samaliev (2011) reported the identification of pathotypes 1, 3 and 4 of the *G. rostochiensis* (Ro1, Ro3, Ro4) and pathotypes 2 and 3 of *G. pallida* (Pa2, Pa3) and found differences in their pathogenicity. The pathotype Ro1 dominated in *G. rostochiensis* -populations, followed by and then Ro3 and Ro4, whereas Pa3predominated in *G. pallida populations*.

Quantitative analysis of live stages of Globodera in soil samples

An assessment of the ecological and physiological status of *G. rostochiensis* and *G. palida* were made on the basis of the cyst viability analysis (Figure 2). Because of the labor-intensive process, data on the presence of male individuals in soil samples are not presented. In Fig. 2 are the values of the mean number of individual life-stages of nematodes.

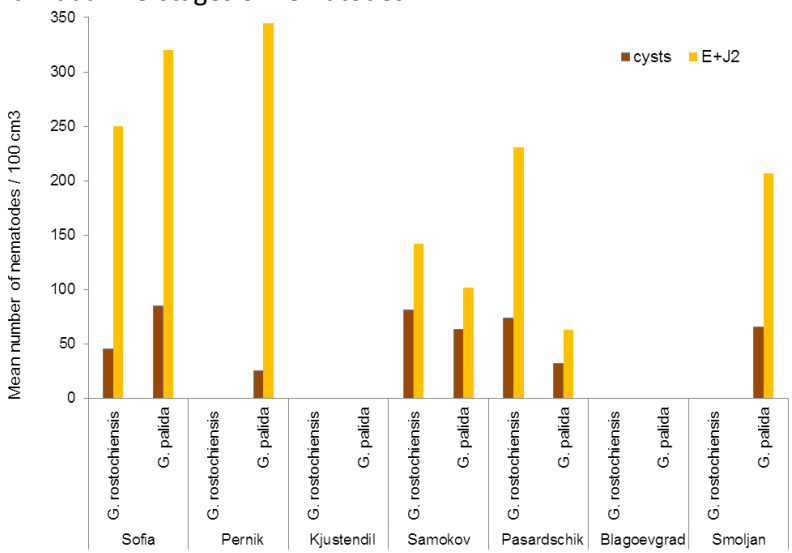

Figure 2. Average number of live eggs and juvenile stages in the cysts of *G. rostochiensis* and *G. pallida* in the observed areas

The mean number of live eggs and juvenile stages J1 in the cysts, as an indication of the extent of distribution, was lower than the incidence of cyst density in the soil. For research period and for the areas where the monitoring was carried out, it could be concluded that the potential risk of spread of infection is decreased. The results obtained by Samaliev (2011) over the period 2006-2008 overlap with our research, but the population density of *Globodera* spp. was lower. For example, for the region of Samokov, the author reports 7-1980 eggs / 100 g of soil, in our studies the number of live eggs and juvenile stages in the cysts of *G. rostochiensis* was 142 E + J2 / 100 cm3, and for *G. palida* - 102 E + J2 / 100 cm3). Considering that the seasonal dynamics of nematodes of Globodera is determined by numerous factors (Ebrahimi et al. 2014). The reasons for the observed differences may be due to the microclimatic characteristics of the area and the meteorological changes in the studied periods. In order to answer this question, the dynamics of the species should be tracked over a longer period of time in close relation to soil and climatic conditions. However, the research was carried out under real production conditions and makes it possible to detect changes in the species composition and density of potato cyst nematodes.

Conclusions

The study revealed the following conclusions:

- Two species of potato cyst nematodes on *G. rostochensis* and *G. pallida* were found in the six potato-growing regions in Bulgaria (Samokov, Pernik, Pazardzhik, Blagoevgrad and Smolyan), with the highest number of cysts were isolated from the Samokov region.
- Rates of spread and density of *Globodera* spesies ranged between regions. *G. rostochiensis* was the most common species in all areas. This species occurred alone in a higher degree than in joint populations of G. pallida.
- The mean number of live eggs and juvenile stages in the cysts as an indicator of the spread of potato cyst nematodes was lower than the frequency of isolation cyst in the soil.

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