

LIFE CYCLE OF THE POTATO GOLDEN CYST NEMATODE (*GLOBODERA ROSTOCHIENSIS*) GROWN UNDER CLIMATIC CONDITIONS IN BELGRADE

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Abstract – The life cycle of a population of the quarantine nematode *Globodera rostochiensis* on the root of susceptible potato variety, Desiree, originating from an infected field (CC Ljubovija no. 413) on the mountain of Jagodnja in the district of Mačva, was studied under experimental conditions in Belgrade in 2002-2003. The golden cyst nematode completed one generation per year in the temperate climate of this region. In 2002, the life cycle lasted 29 days after the penetration of the second stage juveniles into the roots. An adverse effect of high soil temperatures above 25 °C was observed in 2003, influencing the development of the nematode and making the life cycle last two months longer.

Key words: Serbia, quarantine species, golden cyst nematode, *Globodera rostochiensis*, life cycle, potato

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INTRODUCTION

Cysts of the golden nematode (*Globodera rostochiensis* Wollenweber, 1923) and pale nematode (*G. pallida* Stone, 1973), are among the most important pests of the potato, worldwide. In Europe the yield losses caused by these sibling species are about 9% of potato production (Turner and Rowe, 2006). *Globodera rostochiensis* has a larger area of distribution than *G. pallida* and it is the most regulated quarantine nematode species in many countries (Lehman, 2002). This species occurs in temperate and tropical climates. Depending on climate, *G. rostochiensis* completes one or two generations per year (Greco et al., 1988).

In the previous decades, research on the presence of potato cyst nematodes was carried out in Serbia (Krnjaić and Krnjaić, 1991), but it was not until 1999 that positive findings were obtained.

This was the first record of *G. rostochiensis* cysts in soil samples originating from fields used for the production of seed potatoes in Ponikve (Bajina Bašta) and on the mountain of Jagodnja (Krupanj), one of the main potato production regions in western Serbia (Krnjaić et al., 2000, 2002; Radivojević et al., 2001). Due to the lack of other data on *G. rostochiensis*, detailed investigations on this species have been carried out in Serbia since 2001. Research into its distribution was done over the last ten years (Bačić, 2010a) and the effect of potato cultivars on the population of this species was studied (Bačić, 2010b).

This paper reports in detail the results of experiments conducted in 2002-2003 on the life cycle of *G. rostochiensis* under experimental conditions. The aim was to determine the duration of the nematode life cycle and the number of generations per year under local agroecological conditions.

MATERIAL AND METHODS

The experiment on the duration of the different life stages of a population of *G. rostochiensis* from an infected field (CC Ljubovija no. 413) on the mountain of Jagodnja in the district of Mačva, was conducted from May to August in 2002 and 2003, respectively. The trials were carried out in experimental boxes at the Institute of Plant Protection and Environment in Belgrade. Soil weighing about 300 kg was transferred from the infected field. Before planting the susceptible potato variety of potato Desiree, the average density of cysts in 100 ml of the soil (55 cysts per 100 ml of soil, and 40 eggs per cyst or 22 eggs / ml of soil) were determined. The potatoes were planted on 18th May, 2002 and 21th May 2003. In two experimental boxes (1 x 1 m), 25 plants were planted in 10 cm-plastic pots with infected soil, while the same number was planted in a third box with non-infected soil in order to inoculate these plants with newly formed cysts from the first two boxes. Four plants were harvested once a week from the boxes with infected soil. The nematode life stages at each harvest were determined by counting the specimens in 100 ml of soil and 3 g of roots. In order to determine the number of generations, in 2002 newly formed cysts were removed from the roots of plants in infected soil and transferred to pots of potatoes of the same age in non-infested soil. In mid-August, inoculation was performed with 150 newly formed cysts that were transferred into 15 pots (average inoculum of 10 fresh cysts per pot) with non-infected plants of the same age.

For staining the nematodes in root tissue, the roots were chopped into pieces up to 1 cm in length and boiled in a solution of lactophenol with 0.05% acid fuchsin for 3 min (Hooper, 1986). Afterwards, the pieces were washed in running water and transferred into a solution of glycerin and distilled water with a few drops of lactic acid. Dissection of the roots and extraction of nematode specimens at various developmental stages was performed using needles under a stereomicroscope at 40x magnification. The extracted nematodes were fixed, prepared and used

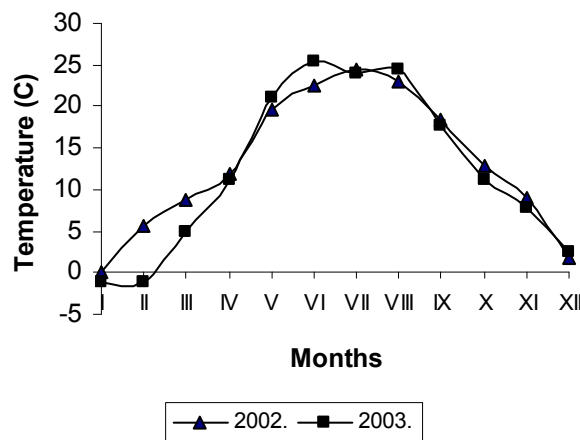


Fig. 1. Average monthly soil temperatures at a depth of 5 cm in Belgrade in 2002 and 2003.

for making temporary glycerin mounts on microscope slides.

Meteorological data for Belgrade in 2002-2003 was provided by the Republic Hydrometeorological Service at Košutnjak in Belgrade. The average monthly soil temperature at the depth of 5 cm during the period from May to September in Belgrade in 2002 ranged from 19.7°C to 23.1°C, and for the same period in 2003 from 21°C to 25.5°C (Fig. 1). The average annual air temperature in Belgrade in 2002 was 13.4°C and the annual amount of rainfall was 589.2 mm (Fig. 2A). The maximum monthly temperature was 23.7°C in July, while the highest monthly rainfall (121 mm) was registered in August. Minor dry periods were recorded in February, March and May. Average monthly temperatures below zero were not registered in 2002, but frosts were reported in January and December. The climate in Belgrade in the second year of research differs from the previous year by a more pronounced dry season (Fig. 2B). Dry periods were registered in March, June and August. The average annual temperature was 12.4°C and the annual sum of precipitation 524.6 mm. The maximum monthly temperature (25.5°C) was in August and the highest amount of rainfall (116.3 mm) in July. Average monthly temperatures below freezing were registered in January and February.

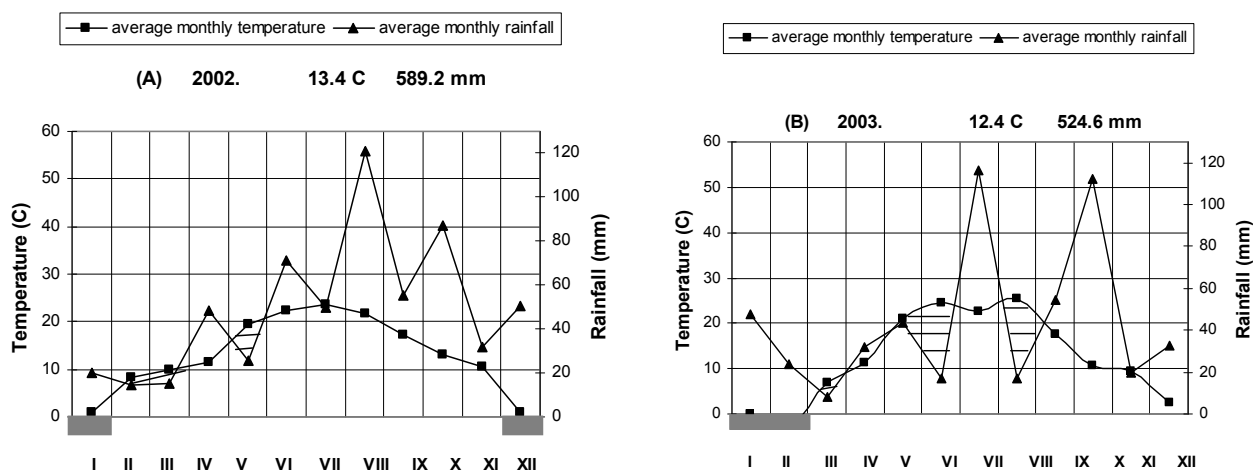


Fig. 2. A i B: Climate-diagram for Belgrade in 2002 and 2003.

RESULTS

During both years of the research, potato shoots were emerged a week after they were planted (25.05.2002 and 28.05.2003) when the first specimens of the second juvenile stage (J2) were registered in the root. At that time, specimens of J2 were present in 100% (Table 1). The percentage of their presence began to fall with the appearance of adult stages. The presence of J2 was registered in 2002 until the end of June, but without an increase of percentage, which would indicate the development of the second generation. In order to prove this, in mid-August artificial inoculation of plants of the same age grown in non-infected soil was done with new cysts. J2 were registered in the root only in the first week after infection. The percentage of J2 fell till mid-June in 2003. No specimen of J2 was registered in the root in July of that year. At the beginning of August, their percentage increased to over 30%. In the middle of the same month, the percentage fell and from then on J2 were not registered in the root.

In 2002, specimens of the third juvenile stage (J3) were registered in the root in the second week (01.06.2002), and in 2003 in the third week (12.06.2003) after the penetration of J2. In 2002, the largest percentage of J3 (more than 57%), was ob-

served a week after they were registered (09.06.2002). Their percentage fell till mid-July 2002 and from then on they were not registered in the root. In 2003, the increase in the J3 percentage was registered at the beginning of July. After that, J3 were not registered in the root.

During both years of research the first specimens of the fourth juvenile stage (J4) and males were found in the third week after the penetration of J2 into the root (09.06.2002 and 12.06.2003). In 2002, a higher percentage of J4 was registered in the fourth week (15.06.2002) and it was 40%. After that, the percentage fell till the end of July 2002. In 2003, two J4 percentage increases were registered - the larger one of 75% in the fifth week after the invasion of J2 into the root (25.06.2002), and a smaller one of 30% (06.08.2003). J4 were being registered in the root till the end of July 2002 and mid-August 2003.

In 2002, the biggest percentage of males (27.6%) was found in the root in the fifth week (22.06.2002) after the penetration of J2. In 2003, two percentage increases in the males were registered - the first one of 40% in the sixth week after the penetration of J2 into the root (02.07.2003), and the second (38%) a month later (06.08.2003). In 2002, males were being found in the root till the end of July, and in 2003 till the end of August.

Table 1. Proportional presence of juvenile and adult stages of *Globodera rostochiensis* in the potato root under the climatic conditions in Belgrade in 2002 and 2003.

Date	J2	J3	J4	Male	Female	Cysts
25.05.2002.	100.0	0.0	0.0	0.0	0.0	0.0
01.06.2002.	91.7	8.3	0.0	0.0	0.0	0.0
09.06.2002.	4.5	57.4	31.6	6.5	0.0	0.0
15.06.2002.	0.0	21.7	43.1	12.7	22.5	0.0
22.06.2002.	2.3	5.4	34.6	27.6	23.1	7.0
29.06.2002.	1.5	6.0	28.7	10.7	34.2	18.9
06.07.2002.	0.0	0.0	9.7	10.9	46.9	32.5
12.07.2002.	0.0	3.3	9.5	7.7	45.0	34.5
20.07.2002.	0.0	0.0	8.6	2.7	33.8	54.9
27.07.2002.	0.0	0.0	2.6	5.2	20.7	71.5
03.08.2002.	0.0	0.0	0.0	0.0	25.4	74.6
28.05.2003.	100.0	0.0	0.0	0.0	0.0	0.0
04.06.2003.	100.0	0.0	0.0	0.0	0.0	0.0
12.06.2003.	76.9	15.4	3.8	3.9	0.0	0.0
18.06.2003.	37.5	12.5	25.0	12.5	12.5	0.0
25.06.2003.	0.0	0.0	75.0	25.0	0.0	0.0
02.07.2003.	0.0	20.0	40.0	40.0	0.0	0.0
09.07.2003.	0.0	0.0	0.0	0.0	0.0	0.0
16.07.2003.	0.0	0.0	0.0	0.0	0.0	0.0
24.07.2003.	0.0	0.0	0.0	0.0	0.0	0.0
30.07.2003.	0.0	0.0	0.0	0.0	0.0	0.0
06.08.2003.	30.7	0.0	30.8	38.5	0.0	0.0
13.08.2003.	25.0	0.0	25.0	25.0	25.0	0.0
21.08.2003.	0.0	0.0	0.0	2.4	48.8	48.8
26.08.2003.	0.0	0.0	0.0	6.1	6.1	87.8

In both years of research females appeared in the fourth week after registering J2 in the root (15.06.2002 and 18.06.2003). In 2002, the percentage of female presence of more than 46% was registered three weeks later (06.07.2002). From then on, the percentage fell. From mid-June till mid-August in 2003 females were not registered in the root. After that, their percentage increased to more than 48%. In 2002, the first mature cysts were extracted on the 29th day after the penetration of J2 into the root (22.06.2002). In the following period, the percentage of cysts increased, and at the beginning of August it was over 74%. In 2003, the cysts were extracted a lot

later (21.08. 2003), and the percentage of their presence at the end of August was over 87%.

When it comes to the presence of juvenile and adult stages in soil in the Belgrade climate, the biggest average number of J2 was registered just as the the plants appeared above ground level at the beginning of June 2002 and at the end of May 2003 (Table 2). After the appearance of males in mid-June, when they were present in the highest number in both years of the research, the number of J2 in soil was reducing. The reduction in their number was more significant in 2003. Cysts were present in the soil in

Table 2. Proportional number of juvenile and adult stages of *Globodera rostochiensis* in 100 ml of soil under the climatic conditions in Belgrade in 2002 and 2003.

Date	J2	Male	Cysts
25.05.2002.	66	0	52
01.06.2002.	157	0	55
09.06.2002.	145	0	49
15.06.2002.	97	32	56
22.06.2002.	60	25	46
29.06.2002.	66	20	51
06.07.2002.	8	9	82
12.07.2002.	25	7	66
20.07.2002.	32	2	58
27.07.2002.	55	0	78
03.08.2002.	28	0	59
28.05.2003.	150	0	73
04.06.2003.	2	0	63
12.06.2003.	20	20	67
18.06.2003.	10	2	62
25.06.2003.	4	4	52
02.07.2003.	2	4	67
09.07.2003.	2	0	63
16.07.2003.	6	0	52
24.07.2003.	5	0	41
30.07.2003.	0	0	65
06.08.2003.	4	5	63
13.08.2003.	4	4	59
21.08.2003.	0	1	71
26.08.2003.	0	2	84

both years of the research since the initial inoculum contained cysts. The biggest number of cysts was registered in the soil at the beginning of July 2002 and at the end of August 2003.

DISCUSSION

Due to long distance between the infected field and the laboratory, the duration of the different life stages of the population of *G. rostochiensis* was observed under experimental conditions in Belgrade. A short reference to the life cycle in such climate conditions was given in a paper on the presence of

this species in Serbia (Bačić, 2010a). The existence of only one generation per year was registered. This could be connected with late planting, the shortened vegetation of the potato and the high soil temperatures. An overlapping of life stages was registered during root and soil sampling in the interval of one week. According to Morris (1971), only one generation develops per year in regions with a temperate climate such as Newfoundland in Canada. One generation of *G. rostochiensis* per year was also registered in Ukraine (Sigaereva et al., 1999), and in Međimurska Županija in Croatia in the period between 2003 and 2005 (Grubišić et al., 2008). It is

possible for the second generation to appear, as was the case in the region of Avezzano in Italy (Greco et al., 1988). This happens when the plants have longer vegetation and if soil temperatures are lower. In such conditions the damage nematodes cause is greater (Greco et al., 1982). A partial generation was registered in cases of shortened potato vegetation, as in the climatic conditions in Bari, where the second generation germinated but is not completed due to high temperatures and the absence of small roots used for feeding (Greco et al., 1988). Similar results were observed in studies of the population of *G. rostochiensis* on the island of Cyprus (Phillis, 1980) where it was noticed that the second generation was not completed. According to Greco et al. (1988), in Bari the first cysts were observed 29 days after the penetration of J2 into the root, which was the same number of days as under the climatic conditions in Belgrade in 2002. According to Alonso et al. (2002), the life cycle of *G. rostochiensis* is shorter in the climatic conditions of the Balearic Islands in Spain due to high temperatures. The results of Spears (1968), Morris (1971) and Brodie (2001) showing a longer life cycle in a population of *G. rostochiensis*, lasting 38-48 days, or 50-58 days due to low soil temperatures in the USA and Canada, were obtained under completely different climatic conditions from those in Belgrade. According to Karapetian et al. (1999), in the former Soviet Union Republic of Armenia *G. rostochiensis* completes one generation per year and it takes 60 days for the first brown cysts to appear on the root. It was recorded that specimens of the second juvenile stage of the second generation were penetrated into the root, but were not able to complete development. In Međimurska Županija in Croatia in the period between 2002 and 2005 it was noticed that the life cycle of this species lasted for 66 or 59 days. Infectious specimens of the second juvenile stage were found in the root during the whole vegetation period, except in the first 20 days after planting (Grubišić et al., 2008).

In our research we observed a negative influence of high soil temperatures on the development of nematodes; this was prominent in 2003. Namely, from 09.07.2003 to 06.08.2003 no life stage was reg-

istered in the root. After that, an increase of the percentage of J2, J4, males and females was registered in August. In July 2003, an extreme rainfall and soil temperatures were registered in Belgrade. Greco et al. (1988) reported that in the south of Italy where the potato is grown as a summer culture, and not an autumn one, when soil temperatures fall under 25 °C, an invasion of specimens of J2 *G. rostochiensis* was registered. We had similar weather conditions in Belgrade during July 2003. Only when soil temperatures fell in August, did J2 invade the root which caused the appearance of the first cysts much later than in 2002. Schluter's findings (as quoted by Turner and Evans, 1998) say that the annual reduction of population, in soil where temperatures over 30 °C were registered, can reach up to 95%. This could explain the sudden reduction in the number of nematodes in our experimental conditions during June and July 2003. In June 2003, a prominent reduction of the number of J2 was registered in the soil while soil temperatures were over 25 °C. This also caused a sudden reduction of their invasion into the root. These facts could maybe explain why no life stage was registered in the root during July 2003. In addition, Turner (as quoted in Turner and Evans, 1998) claims that most J2 enter a diapause during the first year of introduction. This could also be the reason why the intensity of the life cycle of the population of *G. rostochiensis* fell - as the consequence of the adaptation of the population originating from the mountain of Jagodnja to the conditions in Belgrade. According to Turner and Evans (1998), it takes populations of potato cyst nematodes introduced into new regions 2 or 3 years to adapt to different seasons of the growing host plant.

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