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Life Cycle of Potato Cyst Nematode, *Globodera rostochiensis* and Effect of Population Densities on Potato Growth and Yield in Algeria

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ABSTRACT

The present work was aimed to investigate the life cycle and the effect of different initial population densities of *Globodera rostochiensis* on potato yield and growth on the susceptible potato cultivar, Spunta, in Algerian environmental conditions. The length of the life cycle of *G. rostochiensis* differed among growing seasons and was 72 days or 699 $DD_{6.2}$ for potatoes planted in mid-February and 66 days or 496 $DD_{6.2}$ for potatoes planted in early November. A significant reduction in growth and potato yields was observed. The increase of initial Population densities (Pi) of *G. rostochiensis* were associated with a significant reduction in plant growth and potato yields. The maximum yield and plant height reductions were 85 and 75%, respectively, at the highest *Pi* of 512 eggs g^{-1} soil. The final nematode Population density (Pf) increased with the increase of the initial population densities. The Reproductive factor (Rf) initially increased with the increase of *Pi* up to 4 eggs g^{-1} soil and then decreased for Pi≥ 4, suggesting an intraspecies competition. Results contribute to the knowledge of *G. rostochiensis* thermal time requirements in Algerian environmental conditions and can be a valuable tool to develop appropriate potato cyst nematode control strategies considering that the nematode can also cause severe damage and yield losses at very low *Pi* densities.

Keywords: Nematode reproduction, Potato cultivar Spunta, Potato pest, Yield losses.

INTRODUCTION

The Potato Cyst Nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are economically important pests for potato crops, which exist globally in most of the potato cultivated areas (Ngala *et al*., 2015). Both *Globodera* spp. are responsible for yield losses of around 50% in France (Chauvin *et al*., 2008) and up to 80% in Australia (Kooliyottil *et al*., 2017). They are considered as the second most economically important pest of potato in the United Kingdom after *Phytophtora infestans* (late blight) causing yield losses for 26 million euro/year and an additional cost of 10 million euro/year for nematicides (Lord *et al*., 2011). In Bulgaria, the golden potato cyst nematode, *G. rostochiensis* is the main pest causing 80% yield loss (Trifonova, 1995, 2000). In Algeria, PCN were reported for the first time in 1953 (Frezal, 1954). Then, in 1961, the infested areas quickly increased by affecting 33 communes around Algiers (Scotto La Massese, 1961). Subsequently, new populations were discovered in previously uninfected regions, including Ain Defla, Tipaza, Chlef, Mascara and Sétif (National Institute of Plant Protection, 2009; Tirchi *et al*., 2016). Recently, Mezerket *et al*. (2018) revealed the distribution of the PCN in southern Algeria with Informations on the life cycle, population densities and yield losses of *G. pallida* and *G. rostochiensis* under different

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environmental conditions are extremely rare in Algeria. Life cycle of the PCN is well described and generally influenced by temperature. Different numbers of generations were reported from potato grown in different geographical regions according to the temperatures (Greco *et al*., 1988; Renčo, 2007; Bačić *et al*., 2011; Ebrahimi *et al*., 2014). Thus, the temperature sum measured in Degrees-Days (DD) (Trudgill and Perry, 1994; Halford *et al.*, 1999) is an important parameter for predicting PCN development and reproduction.

Several authors used mathematical models to describe the relationship between initial population densities of *Globodera* and potato growth and yield losses (Greco *et al*., 1982; Greco and Moreno, 1992; Hajihassani *et al*., 2013; Maneva and Trifonova, 2015). Understanding the PCN population dynamics in specific agroecosystems and knowing the initial nematode densities at planting will allow predicting any expected yield reductions (Sasanelli *et al*., 2018). According to this information, appropriate control strategies can be selected and implemented to limit nematode damage and improve potato yield (Alonso *et al*., 2011; Hajihassani *et al*., 2013).

The present study was carried out to study the life cycle of *G. rostochiensis* under field in Algerian environmental conditions and to evaluate the effect of different initial population densities of *G. rostochiensis* on plant growth, potato yield and nematode reproduction.

MATERIALS AND METHODS

Plant Material

Potato tubers of the cultivar Spunta, pre-germinated in dark conditions, were used in all the experiments in this study. This cultivar was selected because it was widely used by farmers in Algeria and did not show any level of resistance to potato cyst nematodes, *Globodera* spp.

Nematode Inoculum

The Algerian population of *G. rostochiensis* used in both experiments was obtained from an infested potato field in the El-Oued Region. To obtain nematode inoculum, cysts were extracted from air-dried soil by the Fenwick’s technique (1940). Cysts were then collected and counted under a binocular stereoscope at 40x magnification. The cysts were crushed as described by Seinhorst and Den Ouden (1966) and the number of eggs inside of the cysts was determined.

Microplot Experiment

The experiment on duration of the different life stages of *G. rostochiensis* was carried out in a microplot of 4 m^2, placed in the experimental fields of the National High School of Agronomy (35.23% clay, 23.25% fine silt, 25.24% coarse silt, 10.20% fine sand, 5.78% coarse sand, 0.026% N, 4.89% OM, and pH 7.04). Tubers were sown in Spring (mid-February) and in winter (early November) 2017, at a depth of 12 cm, in two rows spaced 75 cm and 15 cm along the row with a total of 32 tubers. The inoculum for each tuber consisted of 25 *G. rostochiensis* cysts, placed in nylon bags that were added to the soil at the sowing time. During the growing season, the air and soil temperatures (at 10 cm depth), and rainfall were daily recorded (Figures 1, 2, and 3).

Data Collection

The nematode life stages were determined by counting the specimens in 1 g of roots. Root samples were collected at 7-day intervals by harvesting 2 plants at random from the plot at each recording time. The root samples were washed thoroughly and stained with lacto-phenol (28.5% phenol in lactic acid) and acid fuchsin (0.05% in lacto-phenol) to determine developmental stages (Hooper, 1986). The females were counted

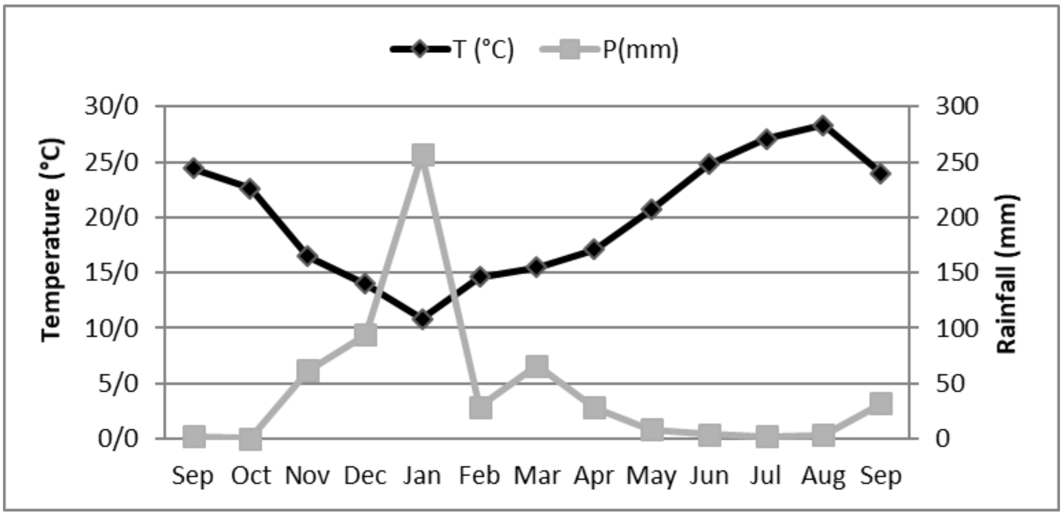

Figure 1. Average values of meteorological variables during the microplot experiments in 2016-2017.

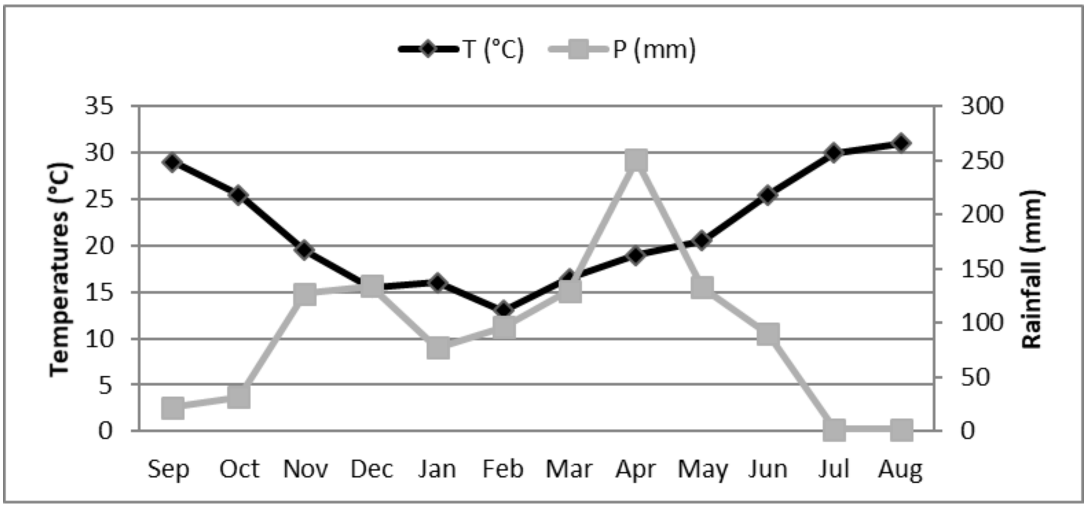

Figure 2. Average values of meteorological variables during the microplot experiments in 2017-2018.

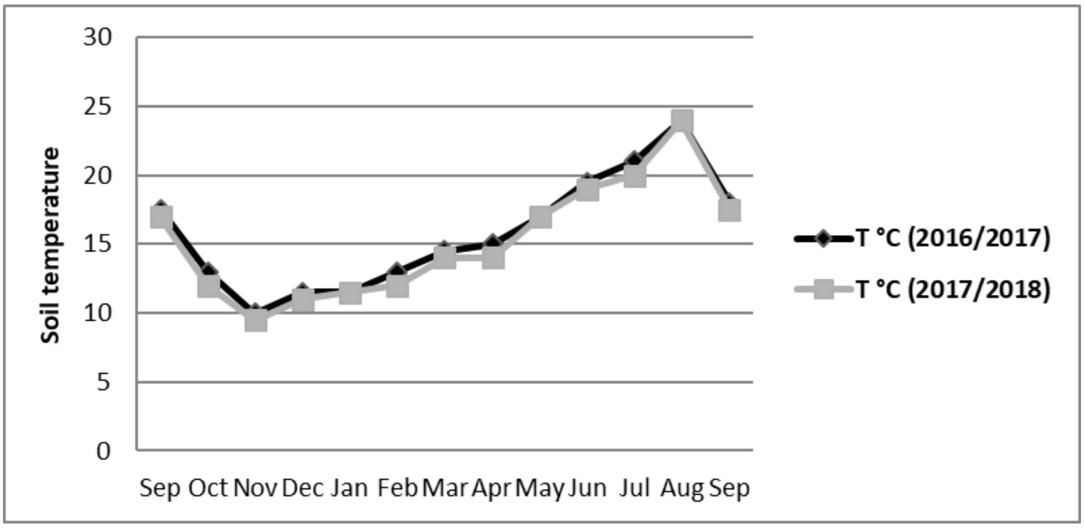

Figure 3. Soil temperature at 10 cm-deep in the microplot experiments in 2016-2017 and 2017-2018.

on the root surface before staining the root. Heat availability was calculated in accumulated Day Degrees (DD) using a basal temperature of 6.2°C for *G. rostochiensis* (Mugniery, 1978).

Pot Experiment

The second experiment on initial population densities was performed in plastic pots (30 cm diameter and 50 cm deep). Each pot, perforated at the bottom for a better drainage, was filled with 8 kg sterilized soil at 80°C for 24 hours (a mixture of 2/3 soil previously indicated and 1/3 breeding soil). Plants were sown in mid-February 2017, with one tuber of uniform size per pot. An appropriate amount of *G. rostochiensis* cysts was placed in tulles and introduced into the soil of each pot to obtain different initial densities from 1 to 512 eggs g^{-1} soil according to a geometric scale (Pi= 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 eggs g^{-1} soil). All pots were arranged in a completely randomized block design with four replications for each population density. Four control pots were not infested. Fertilization was done 15 days after sowing at the rate of 7 g of fertilizer (15% N, 15% P_2O_5, 15% K_2O), for each pot. Standard cropping practices were applied during the growing season (hoeing, weeding, irrigation and pesticide treatments).

Data Collection

For the second experiment, potato plants heights were recorded every three weeks. At harvest (about 100 days later) plants were destroyed and yield determined by weighing potato tubers of each pot (g pot^{-1}). Soil of each pot was air-dried at room temperature and mixed before collecting a 500 g sub-sample to extract the cysts. Cysts were extracted by the Fenwick can (1940). Cysts were then counted and crushed to estimate their egg content and calculate the final nematode population density for each *Pi*. The Reproduction factor (Rf) was calculated as ratio between the final and initial nematode Population densities (Pf/Pi).

Statistical Analysis

Data from the experiments were subjected to analysis of variance ANOVA using the STATISTICA software. Means of treatments were compared by HSD test of Tukey (P= 0.05). Data were also subjected to logarithmic regression analysis.

RESULTS

Microplot Experiment

Different stages of *G. rostochiensis* on potato plants are reported in Table 1 and the average values of meteorological variables during the microplot experiments in 2016-2017 and 2017-2018 are represented in Figures 1 and 2, respectively. During both growing seasons, potato shoots emerged one week after sowing. At that time, second-stage Juveniles (J2) already entered in the root. The first J2 of G. *rostochiensis* were detected in Spring and Winter at 10 and 7 Days After Sowing (DAS), respectively; with an accumulated heat of 83 and 82 $DD_{6.2}$ (Table 1). The soil temperature ranged from 13 to 17ºC and from 10 to 13ºC, for the two considered growing seasons, respectively (Figure 3). In both seasons, third and fourth-stage juveniles (J3+J4) and females were found in roots at 16 and 23 DAS, respectively. When the first females appeared, in Spring and Winter, the accumulated heat were 208 and 199 $DD_{6,}$ respectively. The first cysts of *G. rostochiensis* were found on the roots at 699 DD_6 and 73 DAS in Spring and at 496 DD_6 and 67 DAS, respectively (Table 2). Only one generation was completed per growing season under climatic conditions of Algiers.

Pot Experiment

Effect of *G. rostochiensis* on Growth and Yield of Potato Plants

Emergence of the potato plants was not influenced by the different nematode population densities; although symptoms

Table 1. Number of different stages of *Globodera rostochiesis* recovered from 1 g of roots of potato (cv. Spunta) in the growing seasons (Spring and Winter) 2017/2018.

Date	J2	J3+J4	Female	White female	Cyst
21/02/17	9	-	-	-	-
28/02/17	17	29	-	-	-
07/03/17	3	9	15	-	-
14/03/17	-	8	22	-	-
21/03/17	1	10	16	-	-
28/03/17	-	6	6	-	-
04/04/17	-	3	6	-	-
11/04/17	-	2	4	2	-
18/04/17	-	1	3	1	-
25/04/17	-	1	4	1	1
02/05/17	-	2	3	2	2
09/05/17	-	-	2	2	1
16/05/17	-	-	1	1	3
05/11/17	9	-	-	-	-
13/11/17	12	21	16	-	-
21/11/17	20	14	11	-	-
28/11/17	15	15	18	-	-
06/12/17	10	9	17	-	-
13/12/17	8	7	11	-	-
20/12/17	-	6	6	-	-
27/12/17	2	4	5	3	-
03/01/18	-	3	3	4	-
10/01/18	-	1	4	5	1
17/01/18	-	2	4	3	8
24/01/18	-	1	2	2	5
31/01/18	-	-	1	-	3
07/02/18	-	-	-	-	2

Table 2. Occurrence of *Globodera rostochiensis* developmental stages and degree day accumulations on potato (cv. Spunta).

Developmental stages	Spring		Winter	
	DAS	DD_6	DAS	DD_6
J2	10	83	7	82
J3+J4	17	142	15	147
Female	24	208	23	199
White female	59	533	59	40
Cyst	73	699	67	497

(yellowing and stunting) were observed on plants emerged in inoculated pots with high nematode population densities. The effect of different initial densities (Pi) of *G. rostochiensis* on potato growth and yield was highly significant. Each of the *Pi* studied determined an increase of yield and plant growth losses percentages, compared to the uninoculated control (Pi= 0), by the increase of *Pi* values (Table 3), revealing that the impact of *Pi* was remarkably strong. Moreover, a slight increase in *Pi* caused a significant decrease in the yield and growth of potato demonstrating a high susceptibility of the crop to *G. rostochiensis* attack. Data analysis by logarithmic regression (Figure 4) showed significant negative relationships between potato yield and *Pi* at the end of the plant cycle. The maximum and minimum yield percentage reduction of 85.2 and 16.1% were observed at the highest (512 eggs and J2 g^{-1} of soil) and minimum (1 eggs and J2 g^{-1} soil) *Pi*, respectively (Table 3). Percent plant height reduction, compared to the control, ranged between 17.9 and 75.0% for *Pi* increase from 1 to 512 eggs and J2 g^{-1} soil (Table 3).

Data analysis by logarithmic regression (Figure 5) showed a significant negative relationship between potato yield and initial Population densities (Pi).

Effect of Initial *G. rostochiensis* Population Densities on Population Development and Multiplication Rate

The final Population density (Pf) at the end of the potato crop cycle increased with the increase in *Pi*. Data analysis by linear regression (Figure 6) showed highly significant relationships (R= 0.99) between the initial (Pi) and the final Population densities (Pf). The Reproduction factor (Rf) showed clearly significant results (P< 0.001). The highest *Rf* was determined with an initial population density *Pi*= 4.0 eggs and J2 g^{-1} soil. The Reproduction factor (Rf) increased with the increase of *Pi* (Figure 7)

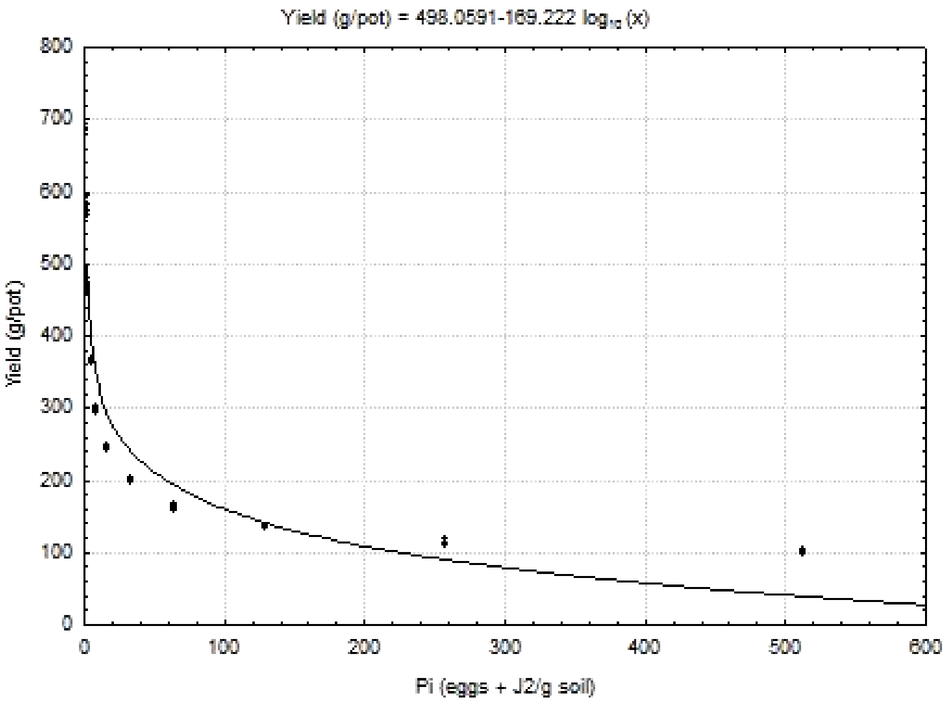

Figure 4. Relationship between potato yield (g pot^{-1}) and initial Population density at sowing (Pi) of *Globodera rostochiensis* (r= -0, 5930; P= 0, 00002).

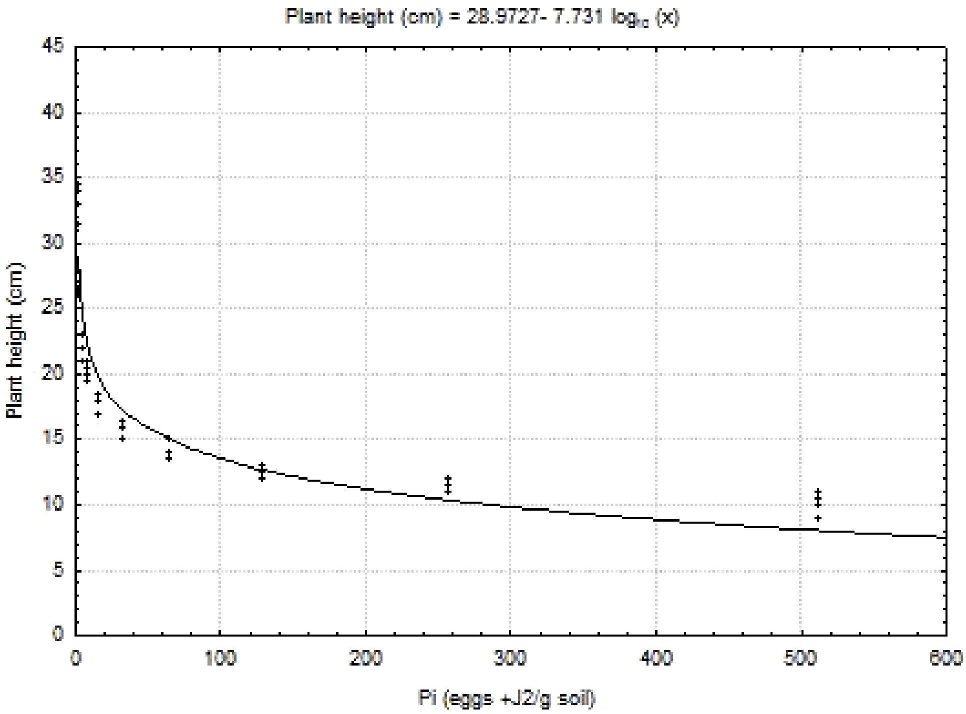

Figure 5. Functional dependence of potato plant height (cm) on the initial density (Pi) of *Globodera rostochiensis* (r = -0, 5949; P= 0, 00002).

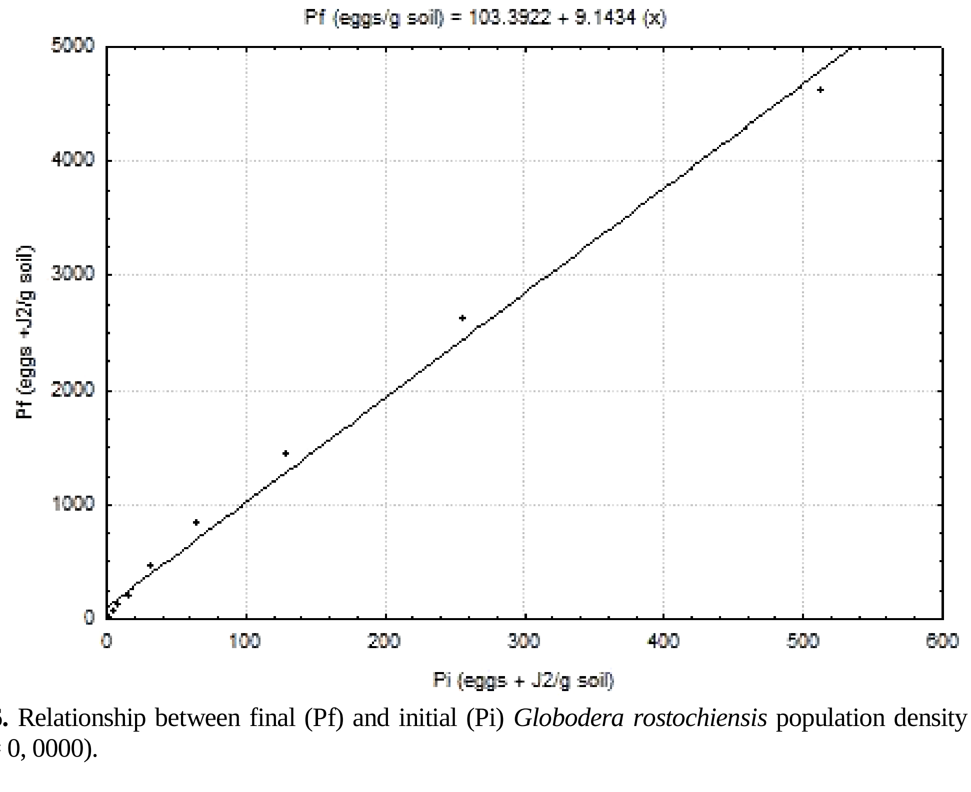

Figure 6. Relationship between final (Pf) and initial (Pi) *Globodera rostochiensis* population density (r= 0, 9961; P= 0, 0000).

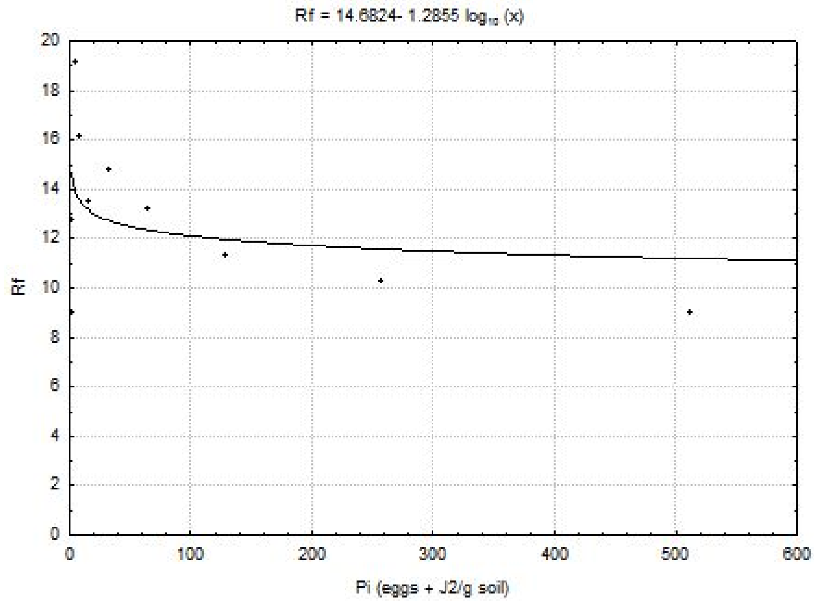

Figure 7. Relationship between Reproduction factor (Rf= Pf/Pi) and the initial *Globodera rostochiensis* Population density (Pi) (r = -0, 1956; P= 0, 5644).

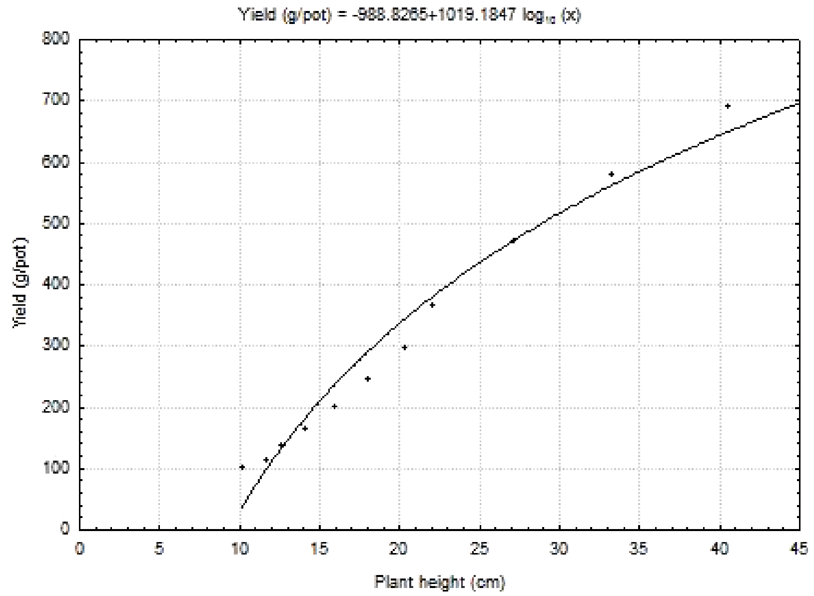

Figure 8. Functional dependence of potato yield (g pot^{-1}) on the plant height (cm) (r= 0, 9962; P= 0, 0000).

until to *Pi*= 4 eggs and J2 g^{-1} of soil. For *Pi* higher than 4 eggs and J2 g^{-1} soil), *Rf* decreased due to intraspecies competition. Potato yield was positively correlated with plant height (Figure 8).

DISCUSSION

The life cycle of different *Globodera* species on potato cultivars has been studied by several authors (Greco *et al*., 1988; Renčo, 2007; Alonso *et al*., 2011; Bacic *et al*., 2011; Ebrahimi *et al*., 2014; Mimee *et al*., 2015). Results of our study confirm that one full generation of *G. rostochiensis* per growing season was recorded.

Our study has shown that the biological cycle from the J2 emergence to the development of new brown cysts in the soil lasted 72 days in Spring and 66 days in Winter. In Cyprus, *G. rostochiensis* completed its life cycle on Autumn and Spring crops at 56 and 63 days after sowing, respectively, when the accumulated DD were 416 and 529 (Philis, 1980). The differences observed were due to the different temperatures during growing season, potato cultivars, and the *G. rostochiensis* pathotype.

In Spring and Winter, first females appeared when the accumulated heat was 208 and 199 DD_6, respectively. Furthermore, the first cysts of *G. rostochiensis* were found on the roots at 699 DD_6 and 73 DAS in Spring. In Winter, the first cysts were found on the roots at 496 DD_6 and 67 DAS. In Italy, to reach the female and cyst stages, respectively, 126 and 168 DD are required in subtropical areas and 275 and 450 DD in temperate regions (Greco *et al*., 1988). Ebrahimi *et al.* (2014) reported 463 DD_4 and 401 DD_6 for *G. pallida* and *G. rostochiensis*, respectively, in microplot and field conditions. The latter values (temperate regions) are close to the accumulated DD required by *G. rostochiensis* in our microplot experiment carried out in Winter (199-496 DD).

The DD values reported by the authors vary with locations and dates of sowing. It should also be noted that different accumulated DD estimates and reported for PCN species might be partially attributed to the methods used to calculate the accumulated DD. Therefore, it is preferable that the authors describe the method for calculating DD values. In this manner, results can be interpreted and compared with each other (Ebrahimi *et al*., 2014).

The effect of different densities of initial populations of different *Globodera* species in potato cultivation has been studied by several authors (Greco *et al*., 1982; Greco and Moreno, 1992; Jiménez *et al*., 2000; Hajihassani *et al*., 2013; Maneva and Trifonova, 2015). Results of these studies confirm that reductions in potato growth and yields are directly related to the initial nematode density in the soil.

Our study has shown that the Algerian *G. rostochiensis* population can lead to a significant decrease in the growth and yield of tubers of cv. Spunta, and the damage increases with the increase of the initial densities of the nematode. According to Seinhorst (1982) and Trudgill *et al.* (1996), losses of tuber yield associated with *Globodera* cyst nematodes attack is due not only to the nematode population density at sowing, but mainly to the penetration and development of juveniles in the root system of the potato crop.

Previous studies (Greco *et al*., 1982; Trudgill, 1985; Greco and Moreno, 1992; Jiménez *et al*., 2000) were carried out on the effect of different initial population densities of *Globodera* on the yield and growth of potato, and the results were processed using the Seinhorst Formula (1965). Results of our research agree with those found in the previously indicated studies. Reduction in potato yield of 20, 50, and 70% at the initial levels of 12, 32 and 128 eggs and J2 g^{-1} soil, respectively, has been observed.

Moreover, results of our study are consistent with those of Hajihassani *et al.* (2013) who reported tuber yield losses at *Pi* 2 and 64 eggs and J2 g^{-1} soil equal to 8.5 and 56% and 9 and 58% for Spunta and Marfona varieties, respectively. Similarly, Maneva and Trifonova (2015) reported a reduction percentage of 47.8 and 73% of plant growth for *Pi* equal to 4 and 128 eggs and J2 gr^{-1} soil, respectively. The significant decrease in plant growth was due to an increased development of *Globodera* J2 in potato root systems, which led, by the histological modifications, to a decrease in water and nutrient uptake in plants (Hajihassani *et al*., 2013). The effect of the initial population on the final density has been confirmed. However, in our study at *Pi*= 2 eggs and J2 g^{-1} soil, a yield loss of 31.9% (in comparison to the uninfested soil) was found, which don't agree with results from Greco *et al*. (1982) who calculated a tolerance limit to *G. rostochiensis* of 1.90 eggs and juveniles/g soil for potato crop. The decrease of *Rf* for $Pi \geq 4$ can be explained by competition for food and space in the high inoculum densities. To know nematode population at sowing time is important to predict yield losses and to organize appropriate control strategies to the problem.

CONCLUSION

In conclusion, the present study establishes relationships between *G. rostochiensis* populations and cumulative temperatures. Knowledge of the day's degree required by the nematodes to develop their life-cycle stages could be used to plan an appropriate harvest time preceding cyst development and minimizing nematode reproduction. Furthermore, results from this study demonstrate that *G. rostochiensis* causes severe yield losses in potato and has the potential to severely impair growth even at very low densities of 1 egg and J2 J2 g^{-1} soil. Continuous testing and monitoring of infested fields to prevent the spread of the potato cyst nematodes is necessary by farmers. Control measures need to be implemented in order to guarantee the lucrative production of potato. A combination of control options such as resistant cultivars, nematicide applications, crop rotations involving PCN resistant cultivars with non-host crops and biological control methods can provide a successful integrated management program against potato cyst nematodes.

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چرخه زندگی نماتد کیست سیب زمینی، *Globodera rostochiensis* و تأثیر تراکم جمعیت آن بر رشد و عملکرد سیب زمینی در الجزایر

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چکیده

هدف این پژوهش بررسی چرخه زندگی و تأثیر تراکم‌های مختلف جمعیت اولیه *Globodera rostochiensis*بر عملکرد و رشد کولتیوارحساس سیب‌زمینی به نام Spunta در شرایط محیطی الجزایر بود. طول چرخه زندگی *G. rostochiensis* در بین فصول رشد متفاوت بود و 72 روز یا 699 DD6.2 برای سیب زمینی های کاشته شده در اواسط فوریه و 66 روز یا 496 DD6.2 برای سیب زمینی های کاشته شده در اوایل نوامبر بود. نتایج کاهش قابل توجهی در رشد و عملکرد سیب زمینی نشان داد. افزایش تراکم جمعیت اولیه (نماتد *G. rostochiensis*) با کاهش قابل توجهی در رشد گیاه و عملکرد سیب زمینی همراه بود. بیشترین کاهش عملکرد و ارتفاع بوته به ترتیب 85٪ و 75٪ در بالاترین عدد Pi با 512 تخم در گرم خاک بود. تراکم جمعیت نهایی نماتد (Pf) با افزایش تراکم جمعیت اولیه افزایش یافت. فاکتور تولیدمثل (Rf) در ابتدا با افزایش Pi تا 4 تخم در گرم خاک افزایش یافت و سپس برای Pi≥ 4 کاهش یافت که نشان‌دهنده رقابت درون گونه‌ای است. این نتایج به آگاهی از نیازهای زمان حرارتی *G. rostochiensis* در شرایط محیطی الجزایر کمک می‌کند و با توجه به اینکه نماتد می‌تواند با تراکم Pi بسیار کم باعث آسیب شدید و تلفات محصول شود، نتایج مزبور می‌تواند ابزاری ارزشمند برای تهیه راهبردهای مناسب کنترل نماتد کیست سیب‌زمینی باشد.