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Estimation of Yield Damage in Potato Caused by Iranian Population of *Globodera rostochiensis* with and without Aldicarb under Greenhouse Conditions

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Abstract

The golden potato cyst nematode, *Globodera rostochiensis* is globally recognized one of the most serious biotic constraints of potato crop. The nematode juveniles infest the roots of plant, which leads to yield loss. The pathogenic potential and reproduction fitness of *G. rostochiensis* on two potato cultivars Marfona and Spunta were determined in two pot experiments with and without the nematicide Aldicarb (G 10%) application. In both experiments, increasing initial population densities (*Pi*) of the nematode in soil was associated with reducing in growth and yield of the plants. These inverse relationships were highly significant and clearly demonstrated using nonlinear regression analysis. The increasing rate of the *Pi* reduced the multiplication rate of *G. rostochiensis* in the plants. In treatments, where nematicide was not applied, increase in the *Pi* levels resulted in yields decreasing exponentially to a maximum tuber yield loss of >50% at 32 to 64 eggs per g soil in cvs. Spunta and Marfona. The nematicide application improved the tuber yield as much as 25% and 30% in cvs. Spunta and Marfona, respectively.

Keywords: *G. rostochiensis*; Multiplication rate; Nematicide; Potato; Yield reduction

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important primary food crops. Among the plant-parasitic nematodes that limit potato production and quality, the potato cyst nematodes (*Globodera* spp.) are the most destructive pests around the world (Marks and Rojancovski, 1998; Hodda and Cook, 2009). Two species of these nematodes, the pale potato cyst nematode, *Globodera pallida* and the golden potato cyst nematode, *G. rostochiensis* are known to parasitize of host plants in many potato-growing systems in European, Asian and American countries (Turner and Evans, 1998) as well as in Australia and New Zealand (OEPP/EPPO, 2004). They are sedentary endo-parasites inducing modified feeding sites called "syncytia" near the vascular tissue in root cells (Sobczak *et al*., 2005). Infection by the potato cyst nematodes leads to a variety of symptoms on both above- and below-ground host plant structures. Above-ground symptoms appear as poor growth, yellowing, wilting, severe stunting and mineral nutrient deficiencies in heavily infested fields (Trudgill, 1980; OEPP/EPPO, 2004), while in soil, root systems may be shorten and branched along with mature females on the root surface at the time of flowering. The yield loss caused by these nematodes on potato production have been estimated ~$70 million per annum in the UK alone (Anonymous, 2010a).

The golden potato cyst nematode, *G. rostochiensis* is widespread in tropical and subtropical climates as well as in temperate regions (Marks and Brodie, 1998) with significant potential to reduce potato tuber yields up to 80% in heavily infested and uncontrolled fields (Spears, 1968). Barker and Koenning (1998) revealed that yield losses incurred in *G. rostochiensis*–susceptible potato averaged 38% (12-76%), compared to 18.3% (12-34%) in resistant potato.

In Iran, potato is one of the most important agriculture crops cultivated most intensively under irrigated conditions throughout the country on 145,000 ha yielding 4.25 million tonnes in 2009 (Anonymous, 2010b). The potato cyst nematodes have been the subject of external quarantine regulations but the cyst-forming nematodes were found on potato roots in Bahar region in Hamadan province in June 2008 for the first time (Gitty and Tanha Maafi, 2009). The morphological and morphometrical characters of second-stages juveniles and perineal pattern of the cysts supported with a molecular test, demonstrated the identity of the golden potato cyst nematode, *G. rostochiensis* (Gitty and Tanha Maafi, 2009). In the infested areas, many patches of poorly growing fields of potato often associated with *Rhizoctonia solani* (AG3) fungus were detected to be infested with intense population levels of the nematode (Gitty *et al*., 2011).

Considering the recent occurrence of *G. rostochiensis* in Iran, the economic impact of *G. rostochiensis* and its relationship with potatoes need to be fully investigated. This

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information is critical for the design of suitable management measures to limit damage caused by *G. rostochiensis* and improve the economic efficiency of potato production. Therefore, the present study examined the relationships between initial populations of *G. rostochiensis* and growth factors of two potato cultivars in pots treated with and without the nematicide *i.e.,* Aldicarb under greenhouse conditions.

Materials and Methods

Two experiments were carried out using plastic pots (25 cm top diameter × 35 cm deep) containing 6 kg soil under greenhouse conditions to determine the damage potential of initial population densities (*Pi*) of the golden potato cyst nematode, *G. rostochiensis* on growth and yield parameters of commercial potato cultivars and the efficacy of nematicide to control the nematode. The treatments consisted of plants inoculated with *G. rostochiensis* only and plants inoculated with *G. rostochiensis* and treated with Aldicarb (Temik, G 10%).

Cysts of *G. rostochiensis* were extracted from soil collected from an infested field of potato at Bahar location in Hamadan province in July 2010. The modified Fenwick-can technique was used to extract cysts from the soil (Stirling *et al*., 1999). The soil used was a mixture of clay, sand and decayed animal manure in the proportion of 2:1:1. Each pot was filled with 5 kg of pasteurized dry soil. Potato seed tubers commonly cultivated in the region cultivars Marfona and Spunta (Super Elite class) were incubated in a growth chamber to produce sprouts approximately 1 cm long and planted in pots (one seed in each pot) after disinfestation by the fungicide Mancozeb. Before inoculation of the nematode, three samples of 50 individual cysts taken at random were crushed to determine the average number of eggs per cyst. The cysts were used as initial population densities including 0, 68, 136, 272, 544, 1088 and 2176 cysts per pot (or 0, 2, 4, 8, 16, 32 and 64 eggs per g soil) for both treatments with and without nematicide application.

For treatments with the nematicide application, 0.0006 kg a.i. per pot (at an equivalent rate of 6 kg a.i. per ha) of Aldicarb were mixed into the soil below the potato tuber at planting. At the same time, cysts of *G. rostochiensis* were placed on the soil surface and covered with an additional 7 cm of soil. All pots were arranged in a completely randomized design with five replications. To deduct position effects in the greenhouse, the place of pots was changed once in each week, allowing the plants to receive equal light. The plants were irrigated twice a week and fertilized two times during the plant developmental stage in each experiment with a 500 mL water-soluble NPK (20:20:20, 0.8% v/v) fertilizer.

At the end of each experiment (about 88 days after planting), fresh shoot weight (g), tuber weight (g), final nematode population (*Pf*) and multiplication rate were measured. The aerial shoots of the mature plants were cut off and root systems in each pot were washed free of soil. Cysts were dislodged from the root systems into a bucket using a strong jet of water. The soil in each pot was air-dried for seven days and then mixed before a 250 g sub-sample was taken to extract cysts by modified sugar centrifugation method (Dunn, 1969). The *Pf* of *G. rostochiensis* was determined by enumerating the total number of cysts and cysts contents collected from both soil and root in each pot using a standard method (Southey, 1970). The multiplication rate was recorded by dividing the *Pf* by the *Pi*.

Data Analysis

All data from both experiments were subjected to analysis of variance (ANOVA) using the SAS statistical program. Data from both experiments were combined and evaluated by nonlinear regression analysis using the curve expert 1.3 software (Hymas, 2005) at $P \leq 0.001$. The best fitting models included Exponential fit (EF), Logistic model (LM), Monomolecular model (MM), Rational function (RF) and Harris model (HM).

Results

Effect of Globodera Rostochiensis on Plant Growth and Yield Parameters

The effect of different initial population densities (*Pi*) of *G. rostochiensis* on fresh shoot weight and tuber yield of potato was significant ($P \leq 0.05$). Results showed a significant suppression of both fresh shoot weight and yield cvs. Spaunta and Marfona with increasing the *Pi* of the nematode as compared to un-inoculated control (Figs. 1 and 2). The regression analysis of the data from both experiments showed significantly negative relationships between the yield of the potatoes and the *Pi* levels at plant maturity. These negative relationships were described by the nonlinear regression models (Fig. 1). The maximum reduction of yield was found with a *Pi* of 2176 cysts per pot (64 eggs per g soil) in the potato cultivars tested.

Reduction in the fresh shoot weight at the greatest *Pi* was generally as much as 66% and 70% in cv. Spaunta and Marfona, respectively. In both experiments, the tuber yield losses of individual plants at a *Pi* of 2-64 eggs per g soil were calculated of 8.5-56% and 9-58% in cvs. Spunta and Marfona, respectively (Fig. 1). The final population (*Pf*) of *G. rostochiensis* increased with increasing the *Pi*, while the multiplication rate (*Pf*/*Pi*) declined as the *Pi* increased. At plants maturity, the multiplication rate of the nematode was greatest at the lowest *Pi* assessed (68 cysts per pot or 2 eggs per g soil) and least at the highest *Pi*. Nonlinear regression analysis clearly showed that the multiplication rate was negatively correlated with the *Pi* of *G. rostochiensis* and the maximum reduction appeared most effectively when the

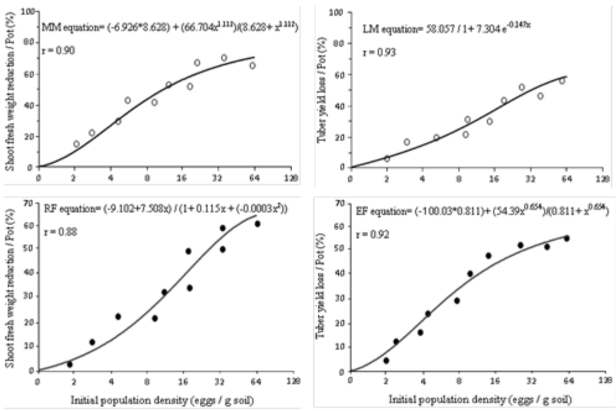

Fig. 1: Fig. 1: Modelled relationships on the effect of *Globodera rostochiensis* on growth and yield parameters of the potato cultivars Marfona (○) and Spunta (●) in pots left untreated with nematicide Aldicarb under greenhouse conditions. Data were combined from two pot experiments (n = 10; $P \leq 0.001$). MM: Monomolecular model, LM: Logistic model, RF: Rational function, EF: Exponential fit

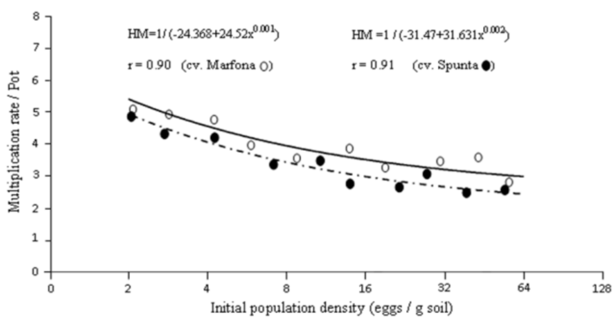

Fig. 2: Relationships between initial population densities (*Pi*) of *Globodera rostochiensis* and multiplication rate (*Pf*/*Pi*) on the different potato cultivars. Data were combined from two pot experiments left untreated with nematicide Aldicarb (n = 10; $P \leq 0.001$). HM: Harris model

high population densities were applied (Fig. 2).

Nematicide Increased Plant Yield and Reduced Multiplication Rate of *Globodera rostochiensis*

The results from two greenhouse experiments were combined for analysis. The results showed that Aldicarb application significantly ($P \leq 0.001$) improved the growth and yield parameters of potato cultivars inoculated by initial populations (*Pi*) of *G. rostochiensis* in comparison with untreated control. Inverse relationships between decreasing fresh shoot weight and tuber yield and increasing *Pi* were significantly correlated and defined using the nonlinear models in both experiments. These reactions were different among the two potato cultivars tested since reduction in the multiplication rate of *G. rostochiensis* due to application of the nematicide was associated with a significant increase in the fresh shoot weight (data not shown). Tuber yield loss at the *Pi* of 64 eggs per g soil was greatest in both cvs. Spunta and Marfona (Fig. 3). In both experiments, the regression analysis clearly showed that the nematicide application improved tuber yield as much as 25% and 30% in cvs. Spunta and Marfona, respectively (Fig. 3).

As the *Pi* of the nematode increased, the *Pf* increased significantly ($P \leq 0.001$) in both experiments treated by the nematicide. In treatments with the nematicide application a significant decline in the multiplication rate of the nematode on the potato cultivars was found. This reduction was minimal at low densities but dropped off gradually at the high densities. However, the multiplication rate was greater than one in potato cvs. Marfona and Spunta at all the *Pi* levels in both experiments (Fig. 4).

Discussion

To provide suitable management strategies for potato cyst nematodes, information is required on the extent of damage they cause on a regional basis (Noe, 1988). The relationship between initial population density of potato cyst nematodes and reduced growth and yield parameters has been established in several studies (Greco and Moreno, 1992; Brodie, 1996) indicating that these soil-borne pathogens are primarily responsible for yield losses or crop rejection observed in mature potato plants. This study showed that Iranian population of the golden potato cyst nematode, *G. rostochiensis* can cause a significant reduction in shoot weight and tuber yield of two cultivars Marfona and Spunta and its damage increased with increasing initial population densities of the nematode. Previous studies clearly highlighted a negative correlation between increasing initial population density of *G. rostochiensis* and yield losses on potato plants (Seinhorst, 1982; Lamondia and Bordie, 1986; Ehwaeti *et al*., 2000). The amount of loss caused by cyst-forming nematodes is closely related to the number of eggs and second-stage juveniles (J2) per g soil. For example, in micro-plots, *G. rostochiensis* reduced yield of potato up to 20, 50 and 70% at initial population levels of 12, 32 and 128 eggs per g soil, respectively (Greco and Moreno, 1992).

In both experiments, *G. rostochiensis* reproduced well on cvs. Marfona and Spunta and theses cultivars were found to be suitable hosts to the nematode. Our modeling clearly showed that predicted yield loss was proportional to the initial populations of *G. rostochiensis* under greenhouse conditions. Tuber yield loss associated with potato cyst nematodes not only is proportional to nematode population density at planting (Seinhorst, 1982; Trudgill *et al*., 1996) but is principally attributed to the penetration and development of the nematode juveniles within the roots. Significant reduction of the fresh shoot weight showed in the present study may be due to increased development of *G. rostochiensis* juveniles within root systems, which leads to decline in the uptake of water and nutrients in the plants. It is known that the yield reduction of potato is affected by

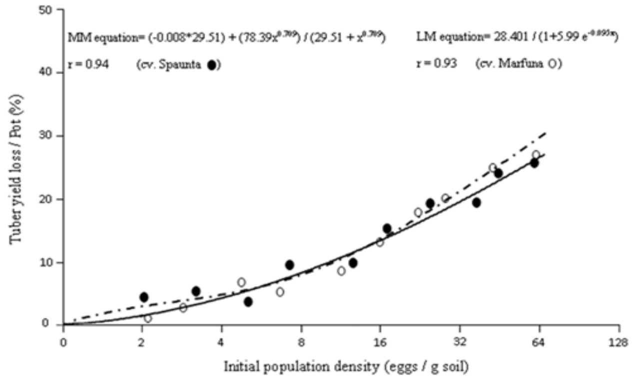

Fig. 3: Modelled relationships on the effect of *Globodera rostochiensis* on the tuber yield of two potato cultivars in pots treated with nematicide Aldicarb. Data were combined from two pot experiments (n = 10; $P \leq 0.001$). MM: Monomolecular model, LM: Logistic model

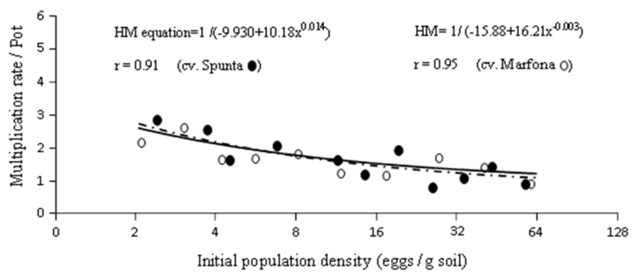

Fig. 4: Relationships between initial population densities (*Pi*) of *Globodera rostochiensis* and multiplication rate (*Pf*/*Pi*) on the different potato cultivars. Data were combined from two pot experiments treated with nematicide Aldicarb (n = 10; $P \leq 0.001$). HM: Harris model

some biotic and abiotic factors such as cultivar and soil type, planting time, climatic conditions (Ehwaeti *et al*., 2000; Hockland, 2002) and most importantly synergistic interactions between these nematodes and fungal soil-borne pathogens (Storey and Evans, 1987; Back *et al*., 2006).

Nematicides have been used to assess the effect of potato cyst nematodes on yields of potato. Regardless of their effects on nematode population, however, nematicides can have stimulatory effects on crop vigour and plant growth (Keer, 2007). Aldicarb, which is used to control potato cyst nematodes, is known to have a strong nematicidial activity against different species of plant-parasitic nematodes on a large number of crops and has given significant yield increases (Smith *et al*., 1991; Moens and Hendricx, 1998). In the current study, using Aldicarb improved significantly tuber yield of both cultivars. The results showed an increase in the tuber yield by 25-30% in cultivars Marfona and Spunta, when initial population densities of *G. rostochiensis* were at the highest of 64 eggs per g soil. The results are similar to those of field and micro-plot experiments conducted with *G. palida* and *G. rostochiensis* by other investigators (Whitehead *et al*., 1994; Minnis *et al*., 2004).

Fernandez *et al*. (2005) revealed that the reproductive fitness of a pathogen, while developing in a host plant is a major component of pathogenicity and thus, is important for the assessment of disease reactions of plants to pathogens. The results of the present work showed that the final population of the nematode increased with increasing initial population, while the multiplication rate decreased as the initial population increased in all treatments. The results showed that the multiplication rate of the nematode in both cvs. Marfona and Spunat was greater than one at all the *Pi* levels. In similar experiments (Lamondia and Bordie, 1986; Ehwaeti *et al*., 2000), the final population of potato cyst nematodes was influenced by initial density and the growing a susceptible cultivar, which is comparable to our results. Arntzen and Bakker (1988) reported that difference in the number of cysts formed on susceptible potato cultivars is depend upon different potato cyst nematode species, different pathotypes and to some extent with different populations of the same pathotype. The definite reasons for reduction in the multiplication rate of *G. rostochiensis* are unknown, but possible explanation may be due to competition for finding host or mate, nutrients and feeding sites among the juveniles, resulting in little multiplication at low population levels.

In both experiments, the final population density of *G. rostochiensis* was reduced by using Aldicarb. The results also showed that application of the nematicide resulted in a significant decline in the multiplication rate of *G. rostochiensis* in two potato cultivars. It is demonstrated that the use of granular nematicides has been effective in reducing potato cyst nematode populations at planting that leads to control (Keer, 2007).

It is now clear that the biggest biotic constraint to potato production in potato fields in hamadan province, Iran is *G. rostochiensis*. Infected small fields in the region do not have sufficient options to rotate potato with non-host plants to avoid significant damage caused by *G. rostochiensis*. Furthermore, since cv. Marfona, which was found a susceptible host for the nematode in the present study, produces good-quality tubers of acceptable yield in a relatively short growing-season, many growers in the infested regions have a strong tendency for the cultivation of this cultivar and apply the nematicide Vapam (Metam-Sodium) at high doses in the soil (personal communication with M. Gitty). This not only may cause a major perennial problem in producing potato through the further spread of *G. rostochiensis* cysts to other regions but also is not very sustainable in terms of the grower (economic costs) and the soil environment.

The economic importance of potato cyst nematodes in the world has increased our current understanding of nematode control strategies. A combination of control options such as eradication programs through continuous testing and monitoring of infested fields to prevent or slow the spread of the nematode cysts, using resistant cultivars or development of genetically resistant varieties, nematicides application, rotations involving resistant and susceptible potato crops with non-host rotation between potatoes and biological control will provide a successful integrated management program for the potato cyst nematodes (Greco *et al*., 1984; Hodda and Cook, 2009).

In Iran, greater attention should be currently drawn to the continuing major threat of *G. rostochiensis* in potato production. Primary management of the disease through preventing of seed tuber transmission to other free-nematode areas and provincial quarantine measures are a must. In addition, highlighting the problem for potato farmers in the nematode-infested areas by encouraging them to use available varieties with durable nematode resistance is necessary.

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