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# Population dynamics of *Globodera pallida* (Nematoda: Heteroderidae) on two potato cultivars in natural field conditions in Balearic Islands, Spain

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## Abstract

*Globodera pallida* is a serious economic pest in worldwide planting of potatoes due to the severe crop losses they can cause. This study aimed to determine the relationship between soil temperature [degree-day accumulation ($DD_4$)] and population development of *G. pallida* under the agroecological conditions of the Balearic Islands, Spain. Population changes were studied in 'Marfona' and 'Maris Peer' potato cultivars for three growing seasons. Differences in the pattern of occurrence of nematode life stages on the two potato cultivars were observed. In Maris Peer trials, second-stage juveniles ($J_2$) hatching occurred about one month after planting (29 and 35 days), $J_2$ root invasion at 57-56 days, and females in roots at 77 days with 540 $DD_4$. In Marfona trials, these nematode stages occurred at 18-31days, 45-52 days and 59-73 days, the last one with 400 $DD_4$. Hence, in Mallorca, *G. pallida* populations require at least 100 $DD_4$ more in Maris Peer early potato than in the Marfona to reach the adult stage**.** Senescence in Maris Peer crops happened before most of the females have become brown cyst, suggesting that in this area the Maris Peer early cultivar could exert trap crop effects. The Marfona crop yield obtained at highest level of nematode infestation may indicate that Marfona seems to be more tolerant to *G. pallida* than Maris Peer. The results contribute to the knowledge of thermal time requirements of *G. pallida* populations under Mediterranean environmental conditions and can be a valuable tool to develop potato cyst nematode control strategies.

**Additional key words**: day-degrees; life cycle; population densities; potato cyst nematode.

## Resumen

### Dinámica poblacional de *Globodera pallida* (Nematoda: Heteroderidae) sobre dos cultivares de patata cultivados en las Islas Baleares (España)

Se estudia la dinámica poblacional de *Globodera pallida* y su relación con las temperaturas del suelo [grados acumulados ($DD_4$)] y con la fenología de la planta hospedadora, en los cultivares de patata 'Marfona' y 'Maris Peer', en las Islas Baleares, España. Se observaron diferencias entre ambos cultivares respecto al patrón de aparición de los estadios vitales del nematodo. En Maris Peer, la eclosión de los juveniles de 2ª edad ($J_2$) se produjo un mes después de la siembra (a los 29 y 35 días), la invasión radical de los $J_2$ 57-56 días después, y la aparición de las hembras en las raíces 77 días después, con 540 $DD_4$. En Marfona, la aparición de los distintos estadios tuvo lugar a los 18-31 días, a 45-52 días y a 59-73 días después de la siembra respectivamente, este último con 400 $DD_4$. Por tanto, en Mallorca, *G. pallida* necesita 100 $DD_4$ más para llegar al estadio adulto en el cultivar Maris Peer que en Marfona. La senescencia del cultivo de Maris Peer se produjo antes de la maduración de los quistes, por lo que en esta región el cultivar de patata temprana Maris Peer podría actuar como cultivo trampa, impidiendo que *G. pallida* finalice su ciclo biológico. El cultivar Marfona presenta una mayor tolerancia a densidades altas del nematodo que Maris Peer. Los resultados obtenidos contribuyen al conocimiento de los requerimientos termales de las poblaciones de *G. pallida* en agrosistemas mediterráneos y pueden tener una importancia fundamental para el diseño de estrategias de control.

**Palabras clave adicionales**: ciclo vital; densidad poblacional; grados acumulados; nematodo formador de quistes de la patata.

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Abbrevations used: $DD_4$ (day degrees accumulation); $J_2$ (second-stage juveniles of *Globodera pallida*); PCN (potato cyst nematodes).

## Introduction

Potato (*Solanum tuberosum* L.) is one of the most important crops in the Balearic Islands, most fields being concentrated on 1,500 ha of irrigated land in the northeastern region of Mallorca Island. Nearly all production is for export. Potato cyst nematodes (PCN) are a serious economic pest in this area due to severe crop losses they can cause and also to the cost of chemical control methods. *Globodera pallida* Stone, was first found in Mallorca in 1966 (Martínez-Beringola *et al*., 1987) and more recently *Globodera rostochiensis* (Woll.) has been found in some potato fields in mixed populations (Andrés *et al*., 2006), but *G. pallida* remains the dominant species (Alonso, 2007). At present, in areas with a long, warm growing season (such as the area studied), the entire crop may be lost in infested fields unless the soil is treated with fumigant nematicides before each potato crop.

PCN species are adapted to a wide range of environments and the seasonal environmental conditions under which potatoes are grown has an influence on their population dynamics and therefore their rate of multiplication and survival (Franco *et al.,* 1998). In temperate regions, these nematodes typically complete one single life-cycle (generation) each year, but in warm soils a partial second generation may appear but activity declines sharply at temperatures above 25°C (Turner and Evans, 1998). An important factor affecting the hatching and development of PCN is temperature and the accumulation of heat energy measured in Day Degrees Centigrade (DD) (Trudgill and Perry, 1994; Halford *et al.,* 1999). Mugniery (1978) established the base temperature for *G. pallida* development at 3.9°C and field studies of *G. pallida* on early potatoes in northern Europe have shown that the amount of heat accumulated above this base temperature during the season has an influence on the rate of development and it is as important as the initial inoculum in determining the final nematode population (Webley and Jones, 1981; Halford *et al.,* 1995).

Understanding of *G. pallida* population dynamics in specific agroecosystems could aid in the development of guidelines for the selection and timing of management and control strategies. This study aimed to determine the relationship between degree-day accumulation and population development of *G. pallida* under the agroecological conditions of the Balearic Islands.

## Material and methods

Trials were conducted in 2001-2003 in four commercial potato fields in Sa Pobla (north-eastern of the island of Mallorca). Each plot was 6.5 m long and 7.5 m wide (10 rows) and was located in the middle of each field, with natural *G. pallida* infestation. Spike rotavation was used to give a uniform distribution of cyst before planting.

Certified seed potatoes of cultivars Maris Peer and Marfona, were planted at a depth of 12 cm, seed spacing of 11 cm and a separation of 75 cm between rows. In this region, Maris Peer is grown as an early cultivar which is planted in November and harvested in March when tubers are still small and loose skinned, while Marfona potato cultivar is grown as a main crop, usually planted in January and harvested in May. The cropping period for Maris Peer was between the 20$^{th}$ November and the 20$^{th}$ April in 2001, and from the 2$^{nd}$ January to the 22$^{nd}$ May in 2002; note that in this year the planting was later than usual due to adverse weather conditions. The Marfona cultivar was planted the 12$^{th}$ January and harvested on the 17$^{th}$ May in 2001 and the 14$^{th}$ January to the 20$^{th}$ May 2003. Standard commercial irrigation, fertilisation and agronomic practices were used to manage the potato crop.

Throughout the growth and development of the crops, measurements and observations were made recording root development, leaf emergence, tuber formation and leaf senescence.

A weather station was installed in each field with a sensor at 10 cm deep. Soil temperature and relative humidity were recorded at half-hourly intervals (Fig. 1). Heat availability was calculated in accumulated day degrees using a basal temperature for *G. pallida* of 3.9°C (Mugniery, 1978).

The initial population (*pi*) was estimated from soil samples of 20 cores (20 × 2.5 cm) taken randomly from the centre of each plot. The cores were combined to give a bulk sample, which was thoroughly mixed. Two subsamples of 100 cm$^3$ of the sieved, dried soil were taken for estimation of the population density, using a Fenwick can for cyst extraction. The cysts were crashed and the eggs and juveniles counted (Shepherd, 1986). Soil samples were taken during growing season from each plot at week intervals following the same methodology for initial population. Hatched juveniles and males were extracted using the centrifugation-flotation method (Nombela and Bello, 1983).

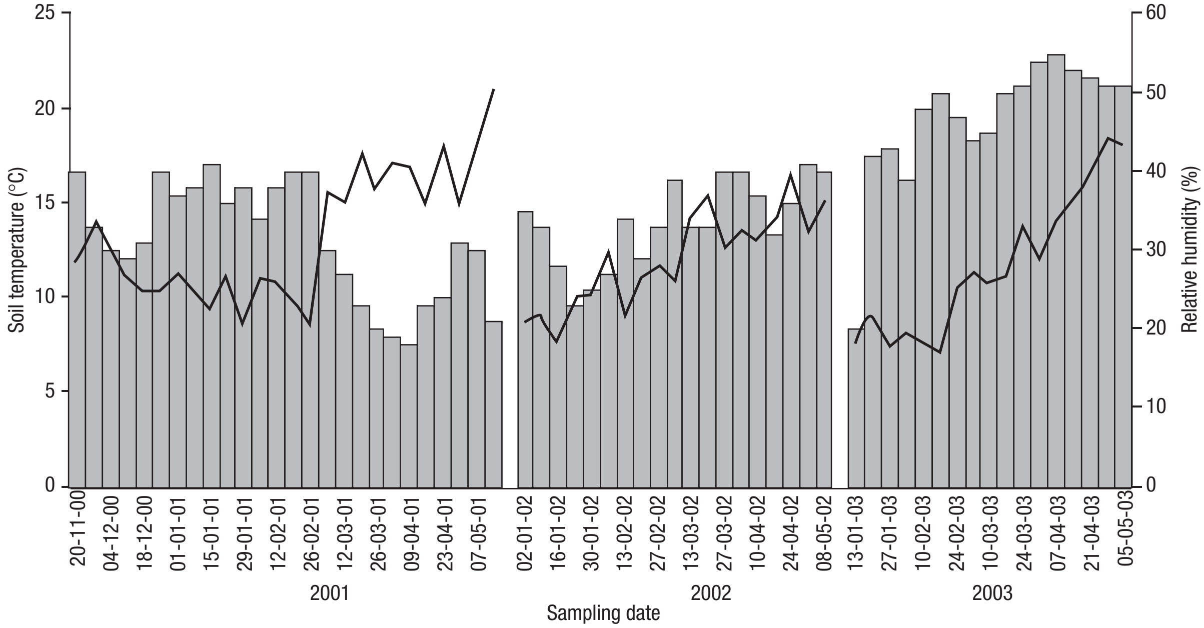


**Figure 1.** Average values of meteorological variables during the field experiments. The soil temperature at 10 cm-deep is represented by a solid line and relative humidity by vertical bars.

Root samples were also taken at 7-day intervals by digging 2 plants at random from each plot every 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 on root surface before staining the root.

Tuber samples were collected at harvest to know the influence of *Globodera pallida* population dynamics in production of Marfona and Maris Peer cultivars. In each plot, four samples were collected by digging the potatoes from 2 m length from the central two rows. Another four tuber samples were taken from plots disinfected by dichloropropene (175 l $ha^{-1}$) as control. Potatoes were washed and weighed to obtain the yield data.

Analysis of variance was performed to determine significant differences between the cultivar yields. Least significant difference (LSD) test was used for the separation of means when there was significant F test ($p < 0.05$). All statistical analyses were performed using the Statistica for Windows program (Statsoft, Tulsa, OH, USA).

# Results

Changes in *G. pallida* population in relation to soil temperature (accumulated day degrees) and plant phenology in Maris Peer and Marfona potato cultivars during the years 2001-2003 are shown in Table 1 and Figure 2.

## Population dynamics in Maris Peer cultivar

Similar population trends were observed in Maris Peer crops during the two growing seasons with only slight differences due to the date of planting. As mentioned above, due to adverse weather conditions in late 2001, Maris Peer was planted in January 2002 instead of the previous November, the intended planting date. The initial population density was 49 and 36 eggs $g^{-1}$ soil in 2001 and 2002 respectively. In this early potato cultivar, second-stage juveniles ($J_2$) were found in the soil approximately one month after planting (29 and 35 days in 2001 and 2002 respectively) (Table 1), coinciding with initial root development (Figs. 2a and 2b) and reached to peak about one month later (64 and 56 days after planting). Soil $J_2$ density was higher in 2001 compared to 2002, with peaks of 56 and 20 $J_2$/100 $cm^3$ soil respectively, coinciding in both cases with plant emergence. The accumulated degrees required to reach this life-cycle stage (hatched $J_2$ in soil) were 234 $DD_4$ (in 2001) and 211 $DD_4$ (in 2002).

Root invasion ($J_2$/root), always correlating with leaf emergence, was observed at 57 and 56 days post-

**Table 1.** Relationships between the first appearance of developmental stages of *Globodera pallida* and degree-day accumulation ($DD_4$) in two potato cultivars, 'Maris Peer' and 'Marfona', in field conditions

| | Maris Peer | | | | Marfona | | | |
|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2002 | | 2001 | | 2003 | |
| | Days | $DD_4$ | Days | $DD_4$ | Days | $DD_4$ | Days | $DD_4$ |
| $J_2$/100 $cm^3$ | 29 | 234 | 35 | 211 | 18 | 86 | 31 | 118 |
| $J_2$/root | 57 | 411 | 56 | 348 | 45 | 257 | 52 | 236 |
| Fem/root | 77 | 541 | 77 | 548 | 59 | 415 | 73 | 400 |
| Yield (t $ha^{-1}$) | 13.25[a] | | 40.42[c] | | 15.72[a] | | 26.87[d] | |
| Yield (t $ha^{-1}$)[1] | 50.6[b] | | 56.32[b] | | 49.74[b] | | 35.9[c] | |

[1] Yield of disinfested field. All yield data with different letters within each potato cultivar were significantly different based on LSD (least significant difference) at $p < 0.05$.

planting (in 2001 and 2002), at 411 and 348 $DD_4$ respectively. The juvenile density in roots peaked (423 $J_2$/root and 285 $J_2$/root in 2001 and 2002 respectively) about one month later, 91 and 84 days after planting.

The first females on root surfaces (fem/root), always coinciding with the appearance of males in the soil, were detected approximately 10 weeks after planting (77 days in both years) at 541 and 548 $DD_4$ (in 2001 and 2002 respectively) and about two weeks before tuber formation. Female density then increased reaching its peak during tuberization, 104 and 98 days after planting, with maximum of 351 and 294 fem/root (in 2001 and 2002 respectively). Adult males and females achieved their maximum densities simultaneously, however the male density observed was always much

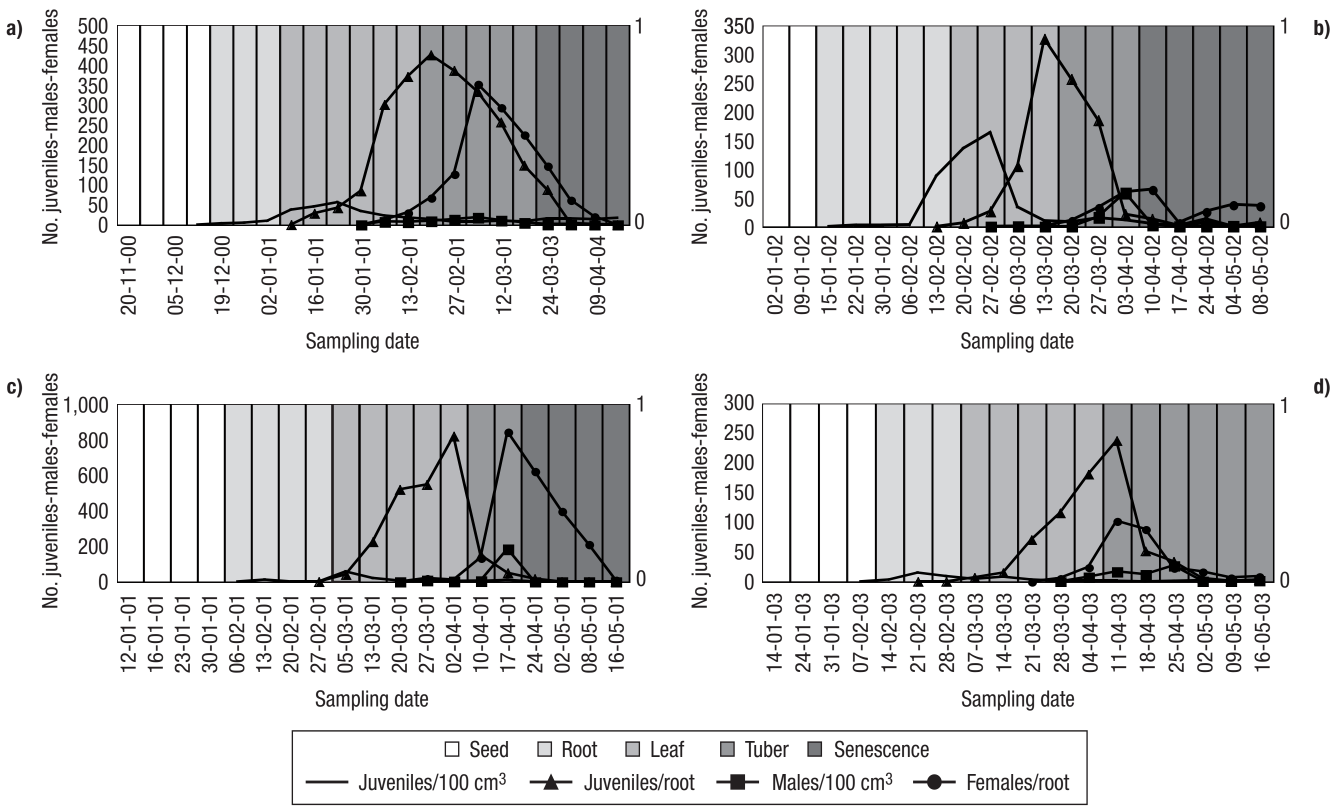


**Figure 2.** *Globodera pallida* population dynamics in soil and root system of 'Maris Peer' and 'Marfona' potato cultivars in natural field conditions: a) Maris Peer, 2001; b) Maris Peer, 2002; c) Marfona, 2001; d) Marfona, 2003.

lower. The number of females on roots was still high when plant senescence began.

Plants were already dead by harvest time in both trials, in spite of the 42 days difference between planting time in 2001 and 2002. At harvest time, a remarkable number of $J_2$ (16 $J_2$/100 cm$^3$) was detected in soil in 2001.

## Population dynamics in Marfona cultivar

In Marfona trial sites the initial infestation levels were very different, high in 2001 with 77 eggs g$^{-1}$ soil and low in 2003 with 5 eggs g$^{-1}$ soil. The first hatched $J_{2s}$ were observed 18 and 31 days after planting in 2001 and 2003 respectively (Figs. 2c and 2d), also coinciding in both cases with the initial root development, accumulated day degrees were 86 and 118 $DD_4$ in 2001 and 2003 respectively (Table 1). The $J_2$ population density in the soil was much higher in 2001 and showed a peak population of 512 $J_2$/100 cm$^3$ soil, coinciding with plant emergence, in contrast to 2003 that the maximum density was 15 $J_2$/100 cm$^3$ (Figs. 2c and 2d). The peaks of $J_2$ population in soil occurred two months after planting (51 days in 2001 and 38 days in 2003).

The first $J_2$ were observed on roots at 45 and 52 days after planting in 2001 and 2003, at 257 and 236 $DD_4$ respectively and always coinciding with leaf emergence. Root infection was very high in 2001 compared with 2003, peaking (1,021 $J_2$/root) at 66 days in contrast to the peak of 236 $J_2$/root at 87 days in 2003.

Females on root surface (fem/root) were found at 59 and 73 days post-planting with 415 and 400 $DD_4$ respectively. In both cases, as observed in Maris Peer trials, this was approximately two weeks before tuber initiation. Females on roots reached their peak (203 fem/root) 95 days after planting in 2001 at the beginning of plant senescence, males appeared at the same time in 2001 and had the maximum numbers one week before, while appeared one week later in 2003 and reached to their maximum two weeks later. Marfona plants died very early (only three months after planting) in 2001, possibly due to the high level of nematode infestation. However in 2003, tubers were harvested four months after planting when the plants were still green and female density on roots showed a lower peak of 102 fem/root. Tubers were harvested earlier due to the bad weather forecast for the next weeks. In both years, a few juveniles were found in the soil at harvest.

## Yield

The yield of two potato cultivars are shown in Table 1. The yield from disinfected plots were significantly higher than those of untreated sites, reaching about 50 t ha$^{-1}$ except Marfona 2003 with only 36 t ha$^{-1}$. In experimental plots, the highest Maris Peer yield was obtained in 2002 with 40.42 t ha$^{-1}$, coinciding with low root infestation level (285 $J_2$/root), in contrast to 13.25 t ha$^{-1}$ obtained in 2001 when nematode density was higher (423 $J_2$/root). Similar production patterns were also observed in 2001 and 2003 in the Marfona cultivar with high (1,021) and low (236) *G. pallida* infestation respectively.

# Discussion

The results showed the influence of accumulated soil temperatures and potato plant phenology in the population dynamics of *G. pallida*. The pattern of occurrence of nematode stages in the early potato crop Maris Peer was markedly different from that in the Marfona crop. In the two growing seasons of the Maris Peer crop, $J_2$ hatching occurred about one month after planting (29 and 35 days respectively), at approximately 200 $DD_4$. Nevertheless, in the Marfona crops the hatched $J_2$ appear rather before, at only 18-31days after planting, and needing about the half degree-day accumulation (86-118 $DD_4$), which advances the successive appearance of the rest of the development stages. In the two growing seasons of Maris Peer, the different nematode development stages occurred at the same time after planting and a similar number of accumulated day-degrees, approximately 540 $DD_4$ were required for female formation, despite of one and a half months difference between the planting dates. Nevertheless, in the Marfona cultivar, which was planted on the same date in 2001 and 2003, some differences between the two growing seasons in terms of the appearance of nematode life-cycle stages were observed. This was probably due to the fact that the 2003 growing season was unusually cooler than in 2001 and plant and nematode development needed more time to reach the same degree day accumulation (400 $DD_4$). Thermal time studies have shown the influence of heat availability in hatch and development of *Globodera* (Trudgill, 1995; Halford *et al.,* 1999); however, no field trial has been carried out with *G. pallida* in Mediterranean agrosystems. Based on the obtained results in the present study, relations between $DD_4$ and dynamics of *G. pallida* population on the two potato cultivars studied could be established and concluded that, in Mallorca Island, *G. pallida* requires at least 100 $DD_4$ more in the early potato Maris Peer than in the Marfona cultivar to reach female stage. Strategies based on degree-day accumulation may be used to mitigate potato crop loss (Pinkerton *et al.*, 1991). Attacking of *G. pallida* to the early potato cultivar could be controlled by early harvesting on the basis of accumulated temperature above the known basal temperature for female development (Webley and Jones, 1981; Halford *et al.,* 1999). Furthermore, knowledge of the degrees day required for nematode development stages could help establish a calendar for in-season nematicides applications (Kim and Yeon, 2001). Consequently, in this potato cultivation region the different thermal times for hatching of *G. pallida* in both cultivars could be considered for nonfumigant nematicide treatments.

The development of PCN life-cycle stages is closely related to potato plant phenology (Greco *et al.,* 1988; Turner and Evans, 1998). In the present study, maximum density of *G. pallida* juveniles in the soil took place during plant emergence, in agreement with the findings of Devine and Jones (2003). Hatching activity in the soil increases rapidly after plant emergence at the same time as root development, stimulated by root leachates (Turner and Evans, 1998; Devine *et al.,* 2001). This feature serves to synchronize the life cycle of the nematode with that of its host (Perry, 1998) since *Globodera* have evolved to respond to host diffusates to ensure $J_2$ maximise their hatch only when host roots are available for invasion (Turner *et al*., 2009). As it has been stated previously, one of the main differences in the population dynamics of *G. pallida* between Maris Peer and Marfona cultivars is the required period to appear the first hatching juvenile in soil which, in addition to temperature, is also mediated by production and diffusion of root exudates. In early potato Maris Peer the root system development takes place later than in Marfona because of the long dormancy of Maris Peer seed in Mallorca potato crops, which can considerably delay the production of exudates. Root structure and development varies between potato cultivars and, therefore, it is reasonable to assume that the production of diffusates at their root tips also varies and, along with it, stimulation of egg hatch (Turner *et al.,* 2009). In all trials of Maris Peer and Marfona cultivars, $J_2$ were detected in the soil after harvesting, probably due to the persistence of hatching factors in the soil. Devine and Jones (2003) have reported that significant levels of hatching factor activity can be detected in the field up to 90 days after potato crop harvesting. These results suggest that under double cropping conditions these juveniles may invade underground host tissue and complete a second generation (Halford *et al.,* 1995). Juvenile invasion was observed when the first leaves appeared and the mature females were observed on root surface simultaneously with tuber formation. The highest number of $J_2$ on plants and females on root surface occurred during tuberization at the time of maximum plant ground cover. The number of juveniles detected on roots was higher than in the soil, except in Marfona during growing season of 2001 when both densities, juveniles in the soil and roots, were considerably higher that affecting plant and nematode development (Trudgill *et al.,* 1975; Schans and Arntzen, 1991; Halford *et al*., 1995) and could induce the early senescence of the crop (Turner and Evans, 1998). In this study, plant senescence in the Maris Peer cultivar always occurred before most of the females had become brown cyst, therefore these immature females do not contribute to soil infestation. In addition potatoes from this cultivar are harvested when they are still small, to be sold as «salad potatoes» in the export market. This indicates that the Maris Peer cultivar, grown in this region as a high-value crop, could exert trap crop effects because *G. pallida* does not have sufficient time to complete its life cycle. The use of early bulking potato cultivars and close monitoring of nematode development may not only permit to reduce PCN field populations, but may also produce a significant yield which would help offset the cost of this alternative method of PCN management (Halford *et al.*, 1999).

As would be expected, yields from disinfested plots were significantly higher than from infested fields. Nevertheless, some differences were found between Maris Peer and Marfona crops. In the Maris Peer trials, all of the plants were dead by harvest time. Both crops had similar durations (150 and 140 days) but the second was planted two months later. During the first year, when the infestation level was very high, the lowest yield was obtained (13.25 t $ha^{-1}$). In 2002 production was significantly greater (40.42 t $ha^{-1}$) coinciding with low nematode density. Marfona crops were planted in January in 2001 and 2003 and were harvested four months later. In 2001, plants were dead at harvest time probably due to the high numbers of juveniles in roots, but plants were still green at harvest time in 2003 and the nematode density in the roots was low. That year, yield could have been greater if crop harvest had been delayed. It could be inferred that yield differences are caused by *G. pallida* density inside roots as long as they were not conditioned by the planting date as Halford *et al.* (1999) pointed out in reference to Maris Peer and other potato cultivars. Furthermore, the analysis of Maris Peer and Marfona yields in 2001, in relation to their respective levels of infestation, showed that Marfona produced a higher yield at higher levels of infestation. This fact demonstrates that, although both have been described like susceptible cultivars (Halford *et al*., 1999; Potato Council, 2008) and have a low tolerance to PCN (Keer, 2006), Marfona seems to be more tolerant to *G. pallida* than Maris Peer in this area. Marfona cultivar produces a large root system and may be tolerant of invasion whilst supporting large populations of nematodes. The crop of tolerant cultivars which are not resistant tend to increase nematode population densities to damagingly high numbers (Trudgill, 1991; Wale *et al.,* 2008) meaning that the use of the Marfona cultivar in this region could give rise to an increase in the population density of *G. pallida* as compared to planting Maris Peer. Nevertheless further studies on the relationship between potato yields and PCN population density in this region are necessary, with having particular interest the influence of threshold level of *G. pallida* for yield reduction in both potato cultivars.

In conclusion, the present study establishes relationships between *G. pallida* populations and cumulative soil temperatures as well as plant phenology of two potato cultivars grown on the island of Mallorca. Knowledge of the degrees days required by the nematode to develop through their life-cycle stages in each potato cultivar could be used to plan harvest time to precede cyst development and thereby minimize reproduction, especially in the case of the Maris Peer cultivar. Furthermore, the monitoring of $DD_4$ and non fumigant nematicide treatment based on $DD_4$ may be an efficient and safe control strategy and serve as an alternative to the use of fumigant nematicide treatment currently used throughout this region.

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