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# Commercial Potato Cultivars Exhibit Distinct Susceptibility to the Root Lesion Nematode *Pratylenchus penetrans*

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**Abstract:** The root lesion nematode *Pratylenchus penetrans* is an important plant-parasitic nematode of potato. In this study, the susceptibility of commercial potato cultivars to *P. penetrans* was assessed. Nematode penetration was evaluated in cultivars Agria, Camel, Kennebec, Laura, Royata, and Stemster at 1, 3, 7, and 15 days after inoculation (DAI) with 750 nematodes/plant, and an egression assay at 3 DAI with 1000 nematodes/plant. Reproduction assays of cultivars Agata, Agria, Camel, Désirée, Dirosso, Kennebec, Laura, Picasso, Royata, and Stemster were performed in 2 L pots inoculated with four *P. penetrans*/g soil and quantified at 60 DAI. Tenue or moderate root cell browning to advanced necrotic areas were observed after nematode penetration, and the number of nematodes/g of root gradually increased with time of infection. A lower number of deposited eggs and nematodes were observed within the roots of cultivar Laura in all assays comparatively to other cultivars. The susceptibility index (SI) was significantly lower in cultivar Laura (0.4–0.6), followed by cultivars Camel and Picasso (0.8–0.9). All remaining cultivars showed SI values above 1. Although the potato susceptibility to the nematode varied among cultivars, no differences on the average number or weight of tubers produced by each plant of inoculated versus non-inoculated plants were detected. Our data reveals that these cultivars have a distinct ability to support the reproduction of *P. penetrans*.

**Keywords:** susceptibility index; *Solanum tuberosum*; egression; penetration; reproduction



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## 1. Introduction

Root lesion nematodes (RLN), *Pratylenchus* spp., are among the world's top 10 plant-parasitic nematodes (PPN), with major worldwide economic impact with ornamental and tree crops (e.g., lily, apple, and cherry orchards) and horticultural plants (e.g., alfalfa, bean, carrot, lettuce, potato, and strawberry) [1,2]. Within this genus, *P. penetrans* [3] Filipjev and Shuurmans Stekhoven, 1941, is considered one the most important species due to its cosmopolitan distribution and wide host range comprising more than 400 plant species [1,4]. Like other RLN species, the migratory *P. penetrans* can enter and leave roots several times during their life cycle, moving actively through the soil and penetrating the host roots for feeding and reproduction [5]. They mainly feed on root hairs and root cortical tissues, using their stylet, which is firstly used as a mechanical tool to probe and puncture the root cell walls [6] and then to secrete various protein effectors, including cell wall-degrading enzymes, produced in three specialized esophageal glands [1,2,7]. Damage caused by *P. penetrans* to potato (*Solanum tuberosum*) fields can be seen as scattered patches of stunted growth plants [4,8]. Belowground, the damage is triggered by direct feeding and migratory activity within the roots [5] and the root symptoms vary from brown to black necrotic lesions, the degradation of epidermal and cortical cells being a typical characteristic of root lesion disease in potato [6], as well as in other type of crops [9]. Infected roots can

have extensive cavities within the cortex cell layers, leading to cell death and collapse, and consequently increasing plant stress [10].

Apart from direct injury to roots and tubers, the concomitant presence of RLN with other pathogens can develop into synergistic disease complexes, such as the potato early dying disease, which results from the interaction of *P. penetrans* with the soil-borne fungus *Verticillium dahliae* [11,12]. Similarly, *P. penetrans* have been also linked to scab, *Streptomyces scabies*, impacting on the marketable quality of potatoes [8].

Common strategies for RLN control include nematicide application/crop rotation/genetic resistance [5]. However, control methods based on nematicides are being restricted due to toxicological/environmental effects [13]. Furthermore, the wide RLN host range limits the effectiveness of cultural practices centered on plant rotation. Identification of natural plant resistance responses to PPN is recognized to play a major role in food security and environmental protection by providing an opportunity to raise yields for major staple crops and to promote sustainable development at a global scale [14,15]. Resistance and tolerance are important attributes to define the dynamics of plant–pathogen interactions. Thus, resistance describes the effects of host genes that restrict or prevent nematode multiplication in a host species, and tolerance reflects the injuries that a nematode can cause on a host plant or the capacity of the plant to withstand, overcome, or reduce the effect of the nematode damage [16–18].

*Pratylenchus penetrans* has been routinely found parasitizing potato in Europe, North America, and Australia [3,8,19–27]. Nevertheless, only a reduced number of commercial cultivars were reported to own some level of tolerance to *P. penetrans* [28–32], with most cultivars tested so far being susceptible to this species. Yield losses of 30–70% have been attributed to the presence of *P. penetrans* in potato fields [8,25,28,31]; for example, population densities of 6000 to 18,000 *P. penetrans*/kg of soil have impacted on the marketable yields of the potato cultivar Sebago by 35–43%, respectively [25]. The yields of Katahdin, Kennebec, and Superior cultivars were also reduced by 20–30% with initial population densities of 380–2000 nematodes/kg of soil [28]. In addition, potato losses of 23–73% were observed in Kennebec, Monona, Norchip, Russet Burbank, Superior, and Yukon Gold with nematode densities ranging from 9800 to 11,500 *P. penetrans*/kg of soil [31]. Holgado et al. [8] reported a potato yield loss of 50% in cultivar Saturna due to *P. penetrans*. Screenings to assess potato susceptibility to *P. penetrans* have been mainly focused on the assessment of nematode final population by measuring the number of RLN found at the harvest in relation with the initial population densities that have been inoculated at planting and/or in relation to root weight [28,31,32]. On the other hand, early egression of *P. penetrans* from roots has been suggested as a reliable measure for identifying resistant and susceptible potato cultivars, as females may egress earlier than males in resistant cultivars [33–35].

Direct molecular detection of *P. penetrans* in potato peels and tuber symptomatology due to the nematode infection has been recently achieved for cultivars Agata, Agria, Camel, Désirée, Dirosso, Kennebec, Laura, Picasso, Royata, and Stemster [36]. However, the susceptibility of these cultivars to the nematode remains unknown, except for cultivar Kennebec, which has been considered susceptible to *P. penetrans* in previous studies [28,31]. The host status of most of these commercial potato cultivars, currently grown in Europe, to *P. penetrans* is not known, reinforcing the need for potato screening against this nematode [37]. The aim of this study was to find out the susceptibility of these potato cultivars to *P. penetrans* through the assessment of penetration, egression, and nematode reproduction. Knowledge on the potential host/non-host status of potato cultivars to *P. penetrans* will permit significant advances towards the management of these resilient pathogens.

## 2. Materials and Methods

### *2.1. Nematode Culture*

The isolate A44L4 of *P. penetrans*, collected from potato roots sampled in the Centre region of Portugal [20], was used in this study. The isolate identity was confirmed by molecular methods using *P. penetrans*-specific primers [20,38]. Nematode cultures deriving

from a single gravid female were propagated on carrot (*Daucus carota*) discs at room temperature (22–25 °C) for 3 months, following Castillo et al. [39]. To obtain the inoculum to set up the assays described below, the infected carrot discs were incubated in sterile water for 24 h.

### 2.2. Plant Material

Certified potato seed from commercial cultivars Agata, Agria, Camel, Désirée, Dirosso, Kennebec, Laura, Picasso, Royata, and Stemster were used in the assays (Table S1). These selected cultivars are commonly grown and commercialized in Europe and are included in the database of European Cultivated Potato (https://www.europotato.org/, accessed on 4 February 2022).

Potato seeds were surface disinfected, washed in tap water with 2–3 drops of Tween 20 (Sigma-Aldrich, Taufkirchen, Germany), immersed in a solution of 70% alcohol for 1–2 min, and finally washed three times with sterilized water. The seeds were then left to sprout until sprouts with ≈2 cm above the tuber were excised. For all the assays (i.e., penetration, egression and reproduction), sprouts were sown to a 1/3 depth into pots filled with an autoclaved soil mixture (50% sand and 50% sandy soil with 10% water content). Potato plants were maintained in a growth chamber adjusted to 21 ± 1 °C, 60% relative humidity, 12 h photoperiod, and watered every 2 days.

### 2.3. Root–Nematode Penetration Assay

Root penetration by *P. penetrans* was assessed in cultivars Agria, Camel, Kennebec, Laura, Royata, and Stemster. Potato sprouts grown for 3 weeks in plastic cups containing ≈0.18 L of soil mixture were inoculated with 750 *P. penetrans* (mixed stages). The nematode inoculum was distributed into four holes made in the soil around the plant. Eight replicates were used for each cultivar in a randomized design and non-inoculated plants used as controls. Roots were harvested at 1, 3, 7 and 15 days after inoculation (DAI). Penetration was assessed by counting the number of nematodes found inside the root tissues stained with fuchsin acid [40]. Mean total numbers of nematodes that have penetrated at each time point were expressed per gram of root and the relative percentage of adults, juveniles, and eggs were also determined.

### 2.4. Nematode Egression Assay

Nematode egression from roots was evaluated in cultivars Agria, Camel, Kennebec, Laura, Royata, and Stemster. Potato explants were transplanted into cone-shaped plastic containers (13.5 cm long × 4 cm Ø) filled with 250 mL of sand. Two holes were made in opposite sides of the plant, and a suspension of 1000 mix stages (juveniles and adults) of *P. penetrans* was added [35]. Treatments were replicated six times in a completely randomized design and non-inoculated plants used as controls. The plants were placed in the growth chamber and at 3 DAI, the root systems from each plant were washed with tap water and transferred into 250 mL flasks filled with 100 mL of tap water (with all the root system covered). The water was collected at each 24 h interval and replaced by fresh water. The collected suspensions were examined every day for four days, and at day six (days five and six combined to analysis), and the total number of juveniles, males, and females quantified. Afterwards, these roots were stained with fuchsin acid to quantity the nematodes that remained inside the roots [40]. The overall percentage of egressed nematodes was calculated based on the number of egressed nematodes at each day in relation to the total number of nematodes that have penetrated the roots (number of egressed nematodes/(number of egressed + stained nematodes within the roots) × 100). The relative percentage of different nematode developmental stages that egressed from the roots at each day was determined for penetration and the female/male ratio.

### *2.5. Nematode Reproduction Assay*

The susceptibility of cultivars Agata, Agria, Camel, Désirée, Dirosso, Kennebec, Laura, Picasso, Royata, and Stemster were evaluated in pot assays. Due to the high number of pots and greenhouse space required, the assay was run in two successive years using similar conditions. In Assay 1, cultivars Agria, Désirée, Kennebec, Laura, Picasso, and Stemster were evaluated, Kennebec being the susceptible control; whereas, in Assay 2, the cultivars Agata, Camel, Dirosso, Kennebec, Laura, and Royata were evaluated. Kennebec and Laura were used as the controls relatively to Assay 1.

These assays were performed in 2 L plastic pots filled with 1.8 L of an autoclaved soil mixture and potato plants were fertilized every 15 days with Nutrea (Genyen-Grow and Protect, Portugal), a water-soluble fertilizer (12% N, 4% P and 6% K), and maintained under the controlled conditions mentioned above. The initial population density (Pi) was 4 nematodes/g soil *P. penetrans* of mixed migratory stages (i.e., a total of 8000 nematodes). Nematodes were applied to four holes that were made in the soil in opposite sides of the plant. Non-inoculated plants were used as the controls. All treatments were replicated six times, and the pots were arranged in a completely randomized design.

At 60 DAI, the plants were harvested, and the following plant growth parameters were recorded: foliage height, foliage fresh weight, root fresh weight, and number and weight of tubers. Nematodes were extracted from the soil using the tray method [41] and quantified to determine the total number of nematodes in the soil in each cultivar. The root systems were washed carefully, fresh weighted, cut into 1 cm pieces and nematodes were extracted after seven days using the Baermann funnel method [42]. Afterwards, nematodes were quantified from suspensions using a stereomicroscope (DM 80; Leica). After extraction (7 days), roots were stained with fuchsin acid to access the number of nematodes that remained inside the roots. The final number of nematodes/g root was calculated as the sum of the extracted plus stained nematodes within roots. Data were analyzed relatively to a susceptibility index (SI): the nematodes/g root of the potato cultivar divided by the nematodes/g of root of the susceptible cultivar control [32].

### *2.6. Statistical Analysis*

Data on plant growth parameters, nematode penetration, egression, and reproduction were checked for normality and homogeneity of variances using the Kolmogorov–Smirnov and Levene tests, respectively. A logarithmic (log (x)) or square root ($\sqrt{x}$) transformation was performed, when necessary, to correct deviations from these assumptions. Data normally distributed were analyzed using analysis of variance (ANOVA), followed by a post-hoc Fisher least significant difference (LSD) statistical test to compare the means, where $p < 0.05$ was considered statistically significant. Statistical analysis of the data was performed using Statistica v. 7 for Windows (StatSoft, Hamburg, Germany).

## 3. Results

### *3.1. Root–Nematode Penetration Assay*

To define the early root symptoms induced by *P. penetrans*, we followed thoroughly the nematode infection process at 1, 3, 7, and 15 DAI, for six potato cultivars (Figure 1). Despite doing the inoculation in the soil, at 1 DAI, motile stages could be found randomly distributed within the roots of the six cultivars. At this time point, we could not verify any preferential zone of penetration, as the nematodes were seen within different regions of the root system of the same cultivar. Nematodes were found in the elongation zone or associated with the root tip, in the junction of lateral branches of the main root, and in different regions of the main root system, as well as in hairy roots. For the remaining time points (3, 7, and 15 DAI), nematodes were within the root cortex layers, as confirmed by acid fuchsin staining of the roots (Figure 1B). During the assay, nematodes have not been observed in the endodermis, in any of the cultivars. Symptoms were not detected in non-inoculated roots, which displayed a typical light, white color (Figure 1A). However, two types of reactions were found on infected roots at 1–3 DAI: 1) a slight to moderate

browning of the punctuated or penetrated cells by nematodes in cultivars Agria, Camel, and Kennebec; and 2) a severe cell browning reaction in Laura, Royata, and Stemster due to the nematode activity (Figure 1). At more advanced time points (i.e., 7 and 15 DAI), darker brown cells to well-advanced necrotic areas were observed in most cultivars.

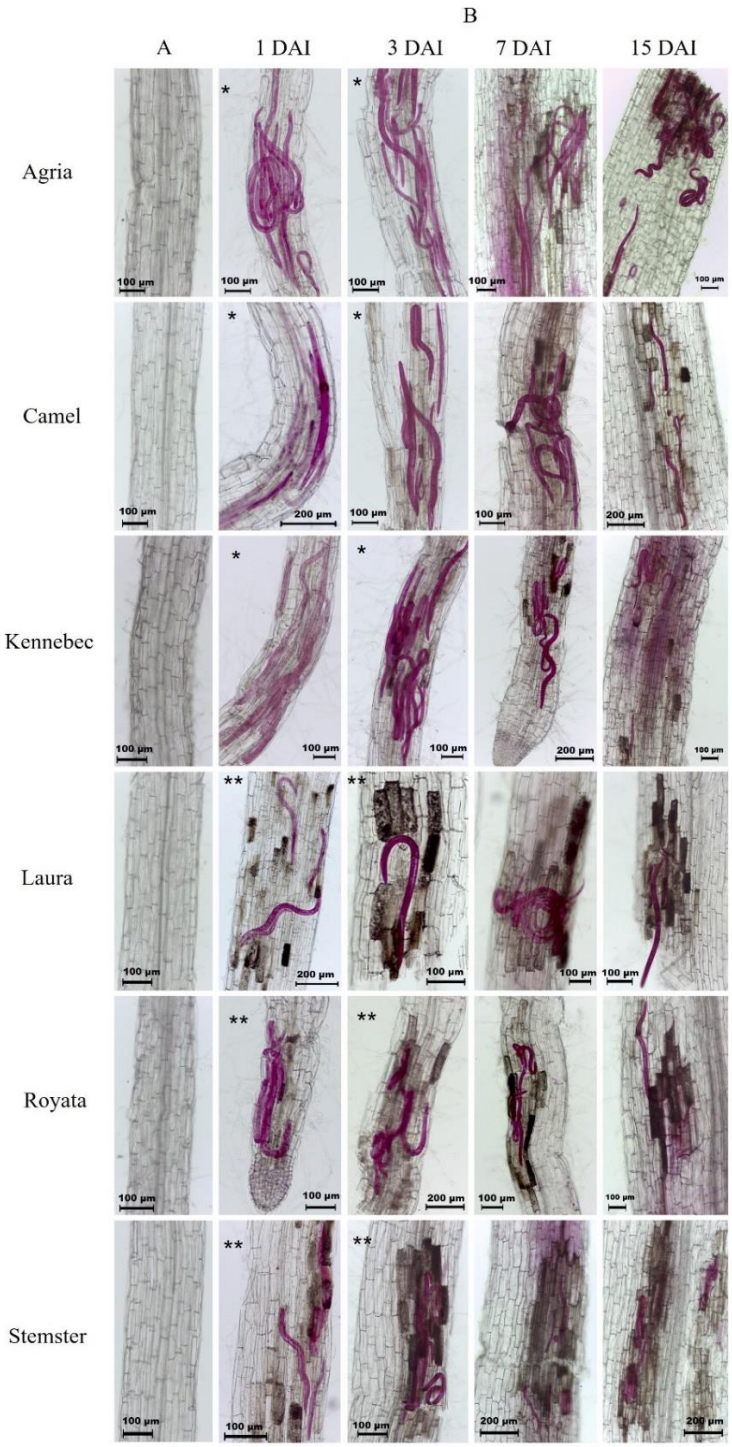


**Figure 1.** Symptoms of early root penetration of *Pratylenchus penetrans* in six cultivars of potato, *Solanum tuberosum*: (**A**) non-inoculated control plants; (**B**) nematode-infected potato plants at 1, 3, 7, and 15 days after inoculation (DAI) with 750 *P. penetrans* mixed stages/plant. Slight to moderate browning of the punctuated or penetrated cells by the nematode in cultivars Agria, Camel, and Kennebec (*), and severe cell browning reaction in Laura, Royata, and Stemster (**) at the early time points 1, 3 DAI.

The total number of nematodes/g root significantly increased over time in all the tested cultivars, ranging from a minimum average number of 5 ± 1.2 nematodes/g of root (cv. Royata) to a maximum of 584 ± 212.4 nematodes/g of root (cv. Agria), from 1 to 15 DAI, respectively (Figure 2). At 15 DAI, statistically significant numbers of nematodes were observed ($p < 0.05$), with cultivars Agria and Camel harboring the highest and lowest average number of nematodes/g of root, respectively (Figure 2).

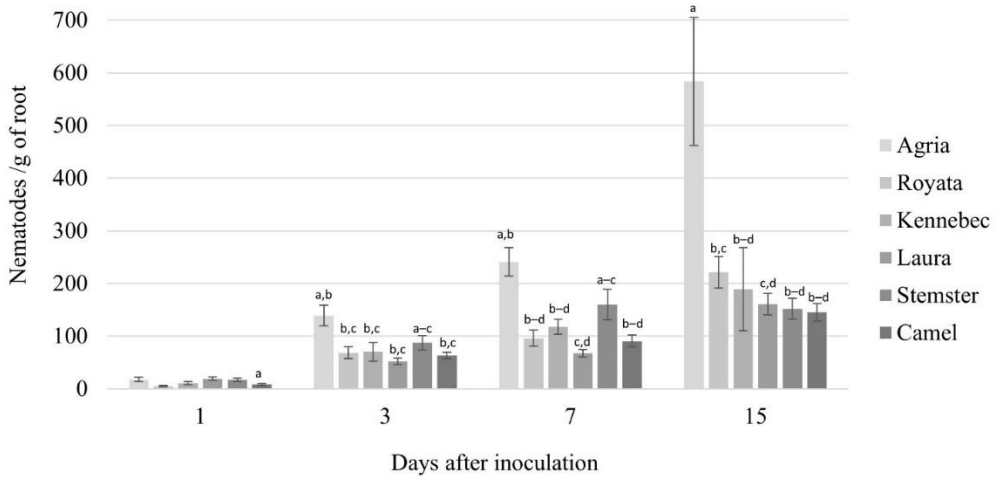


**Figure 2.** *Pratylenchus penetrans*/g of root in potato cultivars Agria, Royata, Kennebec, Laura, Stemster, and Camel at 1, 3, 7, and 15 days after inoculation with 750 *P. penetrans* mixed stages/plant. Data are the means of eight replicates ± SE. Means followed by a different combination of letters differ significantly at $p < 0.05$, according to the Fisher LSD test.

As shown in Figure 1, all motile stages were detected in the roots, as reported in other studies [1,9,10,43]. To have an overview of the relative percentage of nematodes penetrating the roots at these time points of infection, nematodes were separated by developmental stages (i.e., adults, juveniles, and deposited eggs). As nematodes became established within the roots, a relative increase in the number of eggs could be found for all cultivars, with cultivars such as Kennebec showing a two-fold increase, in comparison with cultivar Laura (Figure 3). Cultivar Laura presented the lowest number of eggs (7.3 ± 1.7) at 15 DAI (Figure 3). Most nematodes found inside roots were adults, representing up to 91% at 1 to 7 DAI, and 74% at 15 DAI (Figure S1).

### *3.2. Nematode Egression Assay*

The egression of nematodes from roots (i.e., nematodes exiting the roots) was previously reported as a potential indicator of resistance of certain potato cultivars against *P. penetrans*, with females egressing out of the roots earlier in resistant cultivars [33–35]. Based on our first analyses (Figures 1–3), and to allow a significant number of nematodes penetrate the roots of the different cultivars, plants were harvested at 3 DAI and the egressed nematodes counted from day 1 to day 6 (Figure 4). From the total number of nematodes that have penetrated the potato roots, 30–44% nematodes egressed in five of the cultivars (Agria, Camel, Kennebec, Royata, and Stemster), while in Laura up to 57% of the nematodes egressed from the roots (Figure 4A). The ratio between egressed females and males ranged from 2.5 to 3.9 but were not significantly different among cultivars (Figure S2). Egression occurred mostly within the first 48 h for all cultivars, whereas at the remaining days only a smaller number of nematodes egressed from the infected roots, and the majority remained inside roots (Figure 4B). Considering the results obtained for the penetration assay (Figure 3), the cultivar Laura showed a significant lower number of deposited eggs ($p < 0.05$) in relation to the other cultivars (Figure 4C) following nematode establishment within the roots.

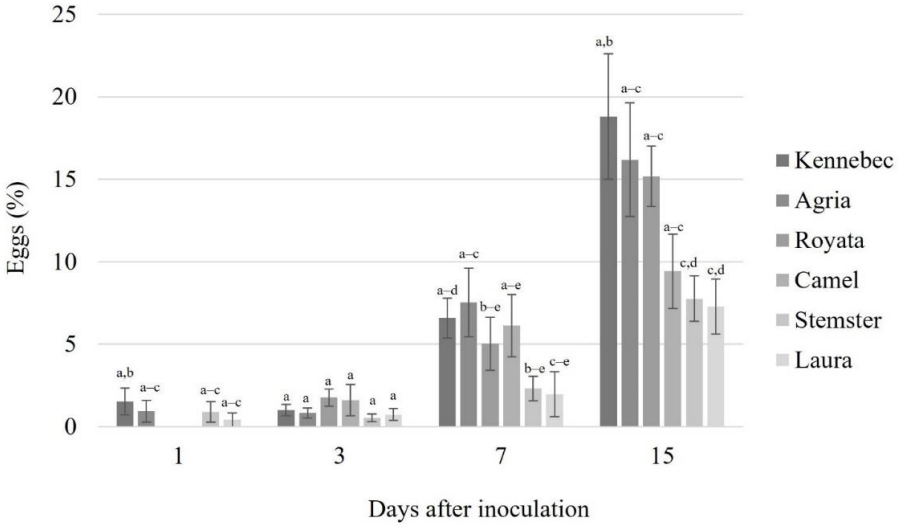


**Figure 3.** Percentage of *Pratylenchus penetrans* eggs deposited relatively to the nematodes inside potato roots of cultivars Kennebec, Agria, Royata, Camel, Stemster, and Laura at 1, 3, 7, and 15 days after inoculation with 750 *P. penetrans* mixed stages/plant. Data are the means of eight replicates ± SE. Means followed by different combination of letters differ significantly at $p < 0.05$, according to the Fisher LSD test.

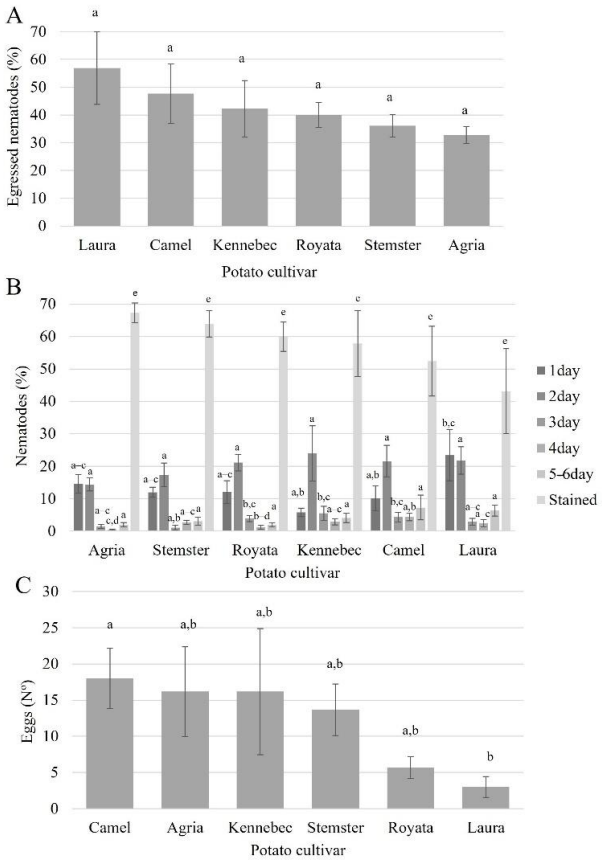


**Figure 4.** Egression of *Pratylenchus penetrans* from potato cultivars, Agria, Stemster, Royata, Kennnebec, Camel, and Laura three days after inoculation with 1000 *P. penetrans* mixed stages: (**A**) percentage of total nematodes (mixed stages) egressed; (**B**) egression throughout six days in relation to those that remain inside the root; (**C**) average number of eggs laid by females in stained roots. Data are means of six replicates ± SE. Means followed by different combination of letters differ significantly at $p < 0.05$, according to the Fisher LSD test.

### *3.3. Nematode Reproduction Assay*

*Pratylenchus penetrans* reproduction was assessed at 60 DAI, to evaluate the susceptibility of each cultivar, allowing the completion of the nematode life cycle. In both assays, no significant differences were found between the foliage parameters and root weight of the inoculated versus non-inoculated plants (Figure 5A–C; Figure 6A–C, Figures S3 and S4)), nor to the number and weight of tubers recovered from each cultivar (Figures 5D,E and 6D,E), except for the foliage height of cv. Agria (Figure 5A). Nevertheless, for some cultivars (e.g., Agria, Désirée, Dirosso, Picasso, Royata, and Stemster), yellowing symptoms of the leaves of nematode-infected plants in comparison to the respective controls were observed (Figures S3 and S5).

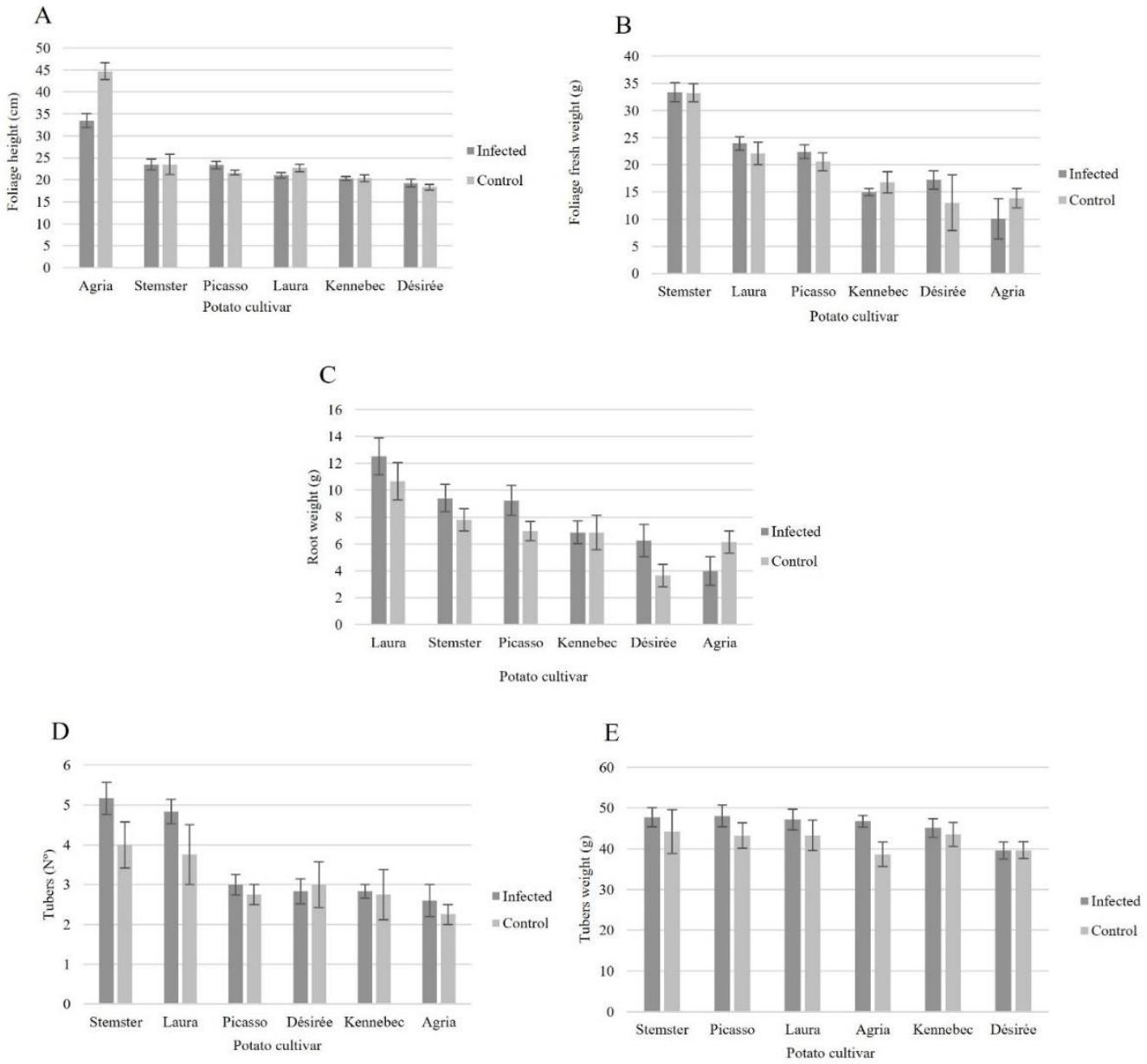


**Figure 5.** Plant growth assessment in Assay 1: (**A**) foliage height; (**B**) foliage fresh weight; (**C**) root fresh weight; (**D**) number of tubers; (**E**) tubers weight. Data are the means of six replicates ± SE. No significant differences were found between foliage, root, and tuber parameters of inoculated versus non-inoculated plants according to the Fisher LSD test, except for the foliage height of cv. Agria.

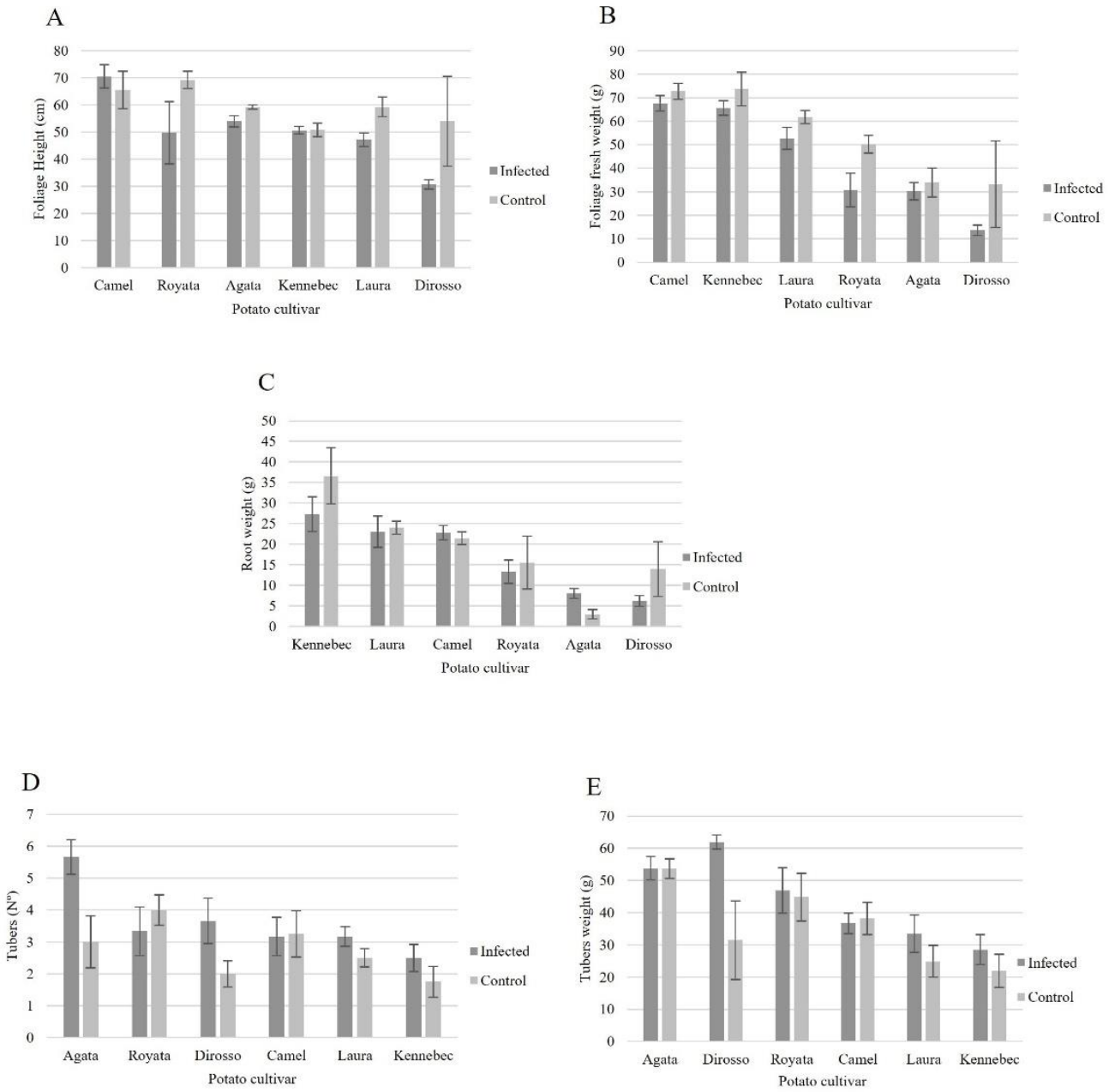


**Figure 6.** Plant growth assessment in Assay 2: (**A**) foliage height; (**B**) foliage fresh weight; (**C**) root fresh weight; (**D**) number of tubers; (**E**) tuber weight. Data are the means of six replicates ± SE. No significant differences were found between foliage, root, and tuber parameters of inoculated versus non-inoculated plants according to the Fisher LSD test.

*Pratylenchus penetrans* is a migratory endoparasite that can enter and leave the roots and remain in the surrounding soil. Therefore, the number of nematodes in both roots and soil were used to assess the host susceptibility of the selected potato cultivars for nematode development (including cv. Kennebec as a known susceptible host). Furthermore, as observed in the egression assay, a greater number of nematodes remained inside roots of all cultivars after nematode extraction, and these were quantified from stained roots. Numbers of nematodes/g root showed that cultivar Laura harbored the lowest number of nematodes/g root (928 ± 59), which is significantly different from Agria (5124 ± 1106) (Figure 7A). In the second assay, the number of nematodes/g root were the lowest in cultivar Laura (694 ± 82), whereas significantly higher densities (2664 ± 358) were recorded for cultivar Dirosso (Figure 7B).

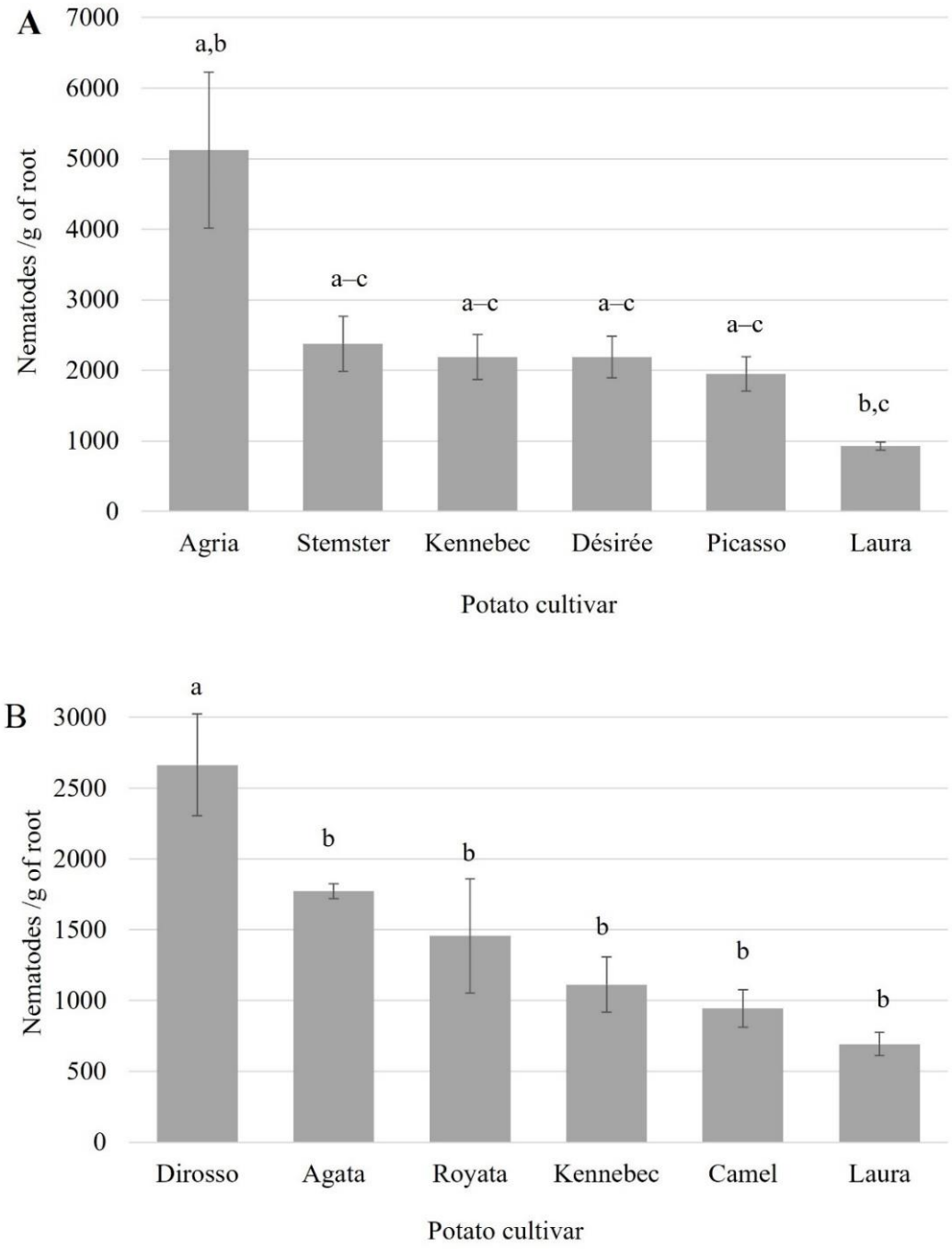


**Figure 7.** Nematodes/g of root on potato, *Solanum tuberosum*, cultivars: (**A**) Agria, Stemster, Kennebc, Désirée, Picasso, and Laura (Assay 1); (**B**) Dirosso, Agata, Royata, Kennebec, Camel, and Laura (Assay 2), 60 days after inoculation with 8000 *Pratylenchus penetrans* mixed stages. Data are the means of six replicates ± standard error. Means followed by a different combination of letters differ significantly at $p < 0.05$, according to the Fisher LSD test.

In Assay 1, the number of eggs laid by females in the cultivar Agria was the highest among all the cultivars (1800 eggs/g root), differing significantly ($p < 0.05$) from Laura, which produced the lowest number of eggs (270 eggs/g root) (Figure 8A). In the second assay, cultivars Agata and Dirosso presented the highest number of eggs (450 and 350, respectively), being statistically different from cultivar Laura (130/g root) (Figure 8B).

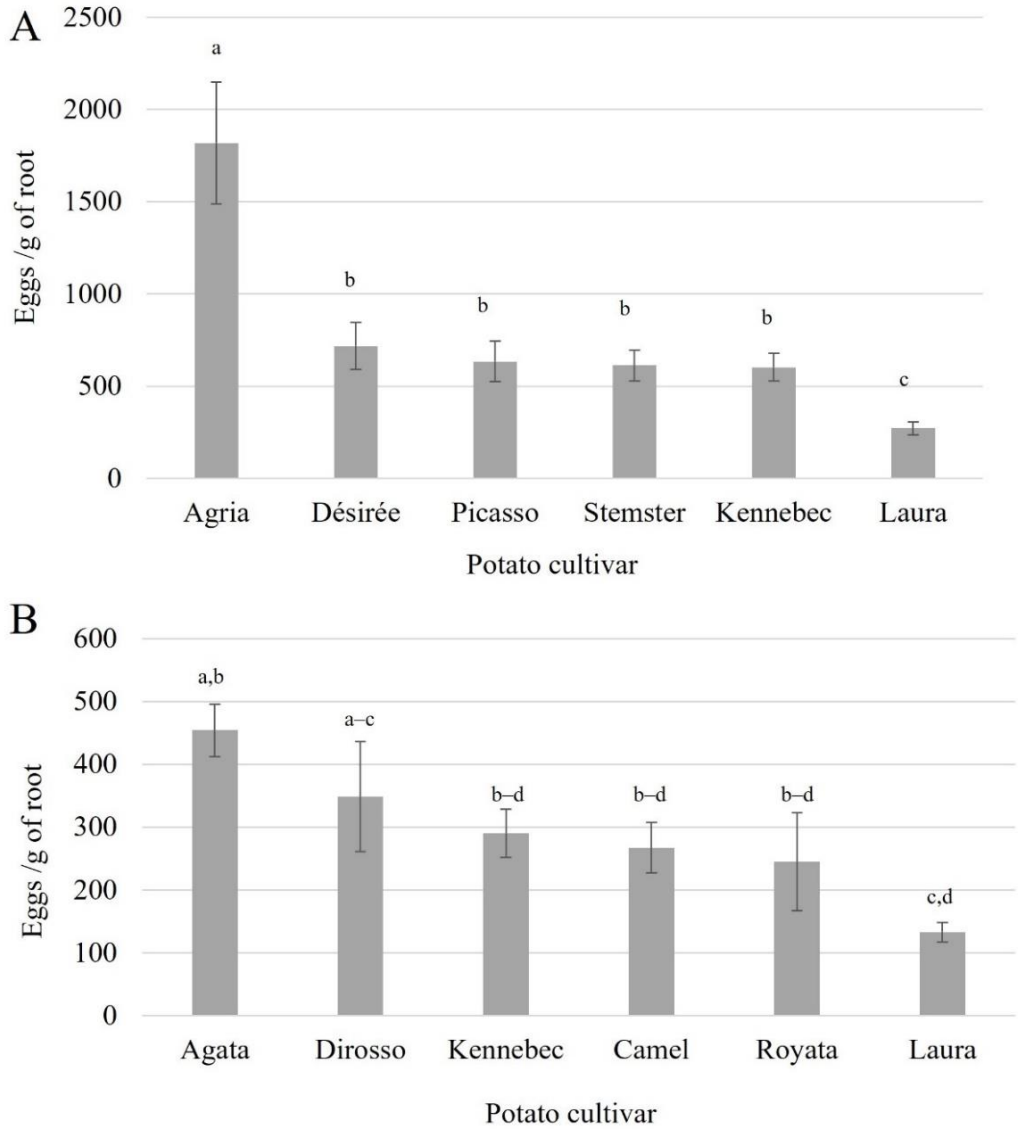


**Figure 8.** Eggs/g of root of on potato, *Solanum tuberosum*, cultivars: (**A**) Agria, Désirée, Picasso, Stemster, Kennebc, and Laura (Assay 1); (**B**) Agata, Dirosso, Kennebec, Camel, Royata, and Laura (Assay 2), 60 days after inoculation with 8000 *Pratylenchus penetrans* mixed stages. Data are the means of six replicates ± standard error. Means followed by different combination of letters differ significantly at $p < 0.05$, according to the Fisher LSD test.

The results obtained in this assay were then translated into a susceptibility index (SI), calculated according to Brodie and Plaisted [32], considering the cultivar Kennebec as a known susceptible genotype [28,31]. There were significant differences among the cultivars screened within both assays ($p < 0.05$) (Table 1). Cultivar Laura had the lowest SI (0.4 and 0.6) in both assays, followed by Camel and Picasso with SI values of 0.8 and 0.9, respectively. Remarkably, all remaining cultivars showed SI values above 1, with Agria reaching 2.3 and Dirosso 2.4, suggesting a higher level of susceptibility to *P. penetrans*. Nevertheless, although potato susceptibility to the nematode varied among cultivars, no differences on the average number or weight of tubers produced by each plant were perceived when compared with the non-inoculated plants (Figure 5D,E and Figure 6D,E) Observation of stained roots at 60 DAI revealed that all cultivars inoculated with *P. penetrans* exhibited necrotic lesions, often displaying severe and extensive areas of necrotic tissue.

**Table 1.** Susceptibility index (SI) of potato cultivars 60 days after inoculation with 8000 *Pratylenchus penetrans* mixed stages. SI followed by different combination of letters differ significantly at $p < 0.05$, according to the Fisher LSD test.

| Assay | Potato Cultivar | Susceptibility Index (SI) |
|---|---|---|
| 1 | Laura | 0.4 $^{b,c}$ |
| | Picasso | 0.9 $^{a,b,c}$ |
| | Kennebec | 1.0 $^{a,b,c}$ |
| | Désirée | 1.0 $^{a,b,c}$ |
| | Stemster | 1.1 $^{a,b,c}$ |
| | Agria | 2.3 $^{a,b}$ |
| 2 | Laura | 0.6 $^{b}$ |
| | Camel | 0.8 $^{b}$ |
| | Kennebec | 1.0 $^{a}$ |
| | Royata | 1.3 $^{b}$ |
| | Agata | 1.6 $^{b}$ |
| | Dirosso | 2.4 $^{b}$ |

## 4. Discussion

To sustain a growing world population, food production is required to increase. However, plant pathogens pose a continuous and serious threat towards this goal due to their significant impact on reducing crop yield and quality. Potato is one of the most important food crops in the world [44,45], and is often critically damaged by plant-parasitic nematodes such as the root lesion nematode, *P. penetrans* [46,47]. The ability to manage this nematode using natural resources is critically limited due to the absence of effective resistant cultivars. Although previous studies assessed host performance against several potato cultivars, only a reduced number of cultivars have shown some level of resistance or tolerance to *P. penetrans* [28–32]. The economic threshold of potato to *P. penetrans* is documented as 1–2 nematodes g/L soil [1], and a decrease in the number of nematodes/g of root with time indicates that population development might be suppressed in resistant/tolerant cultivars [32]. In this study, we validate the host susceptibility of a variety of commercial potato cultivars often selected for potato cultivation in Europe, as no reports are available on the response of these cultivars to *P. penetrans*.

The invasion and establishment process taken by *Pratylenchus* spp. is composed by the recognition of the host plant, followed by inter- and intra-cellular root migration of the nematodes [5,6,48]. Nematode penetration gradually increased with time after inoculation for all the herein-tested cultivars. Our results are consistent with the hypothesis that the initial penetration of root lesion nematodes is not affected by the level of susceptibility/resistance of the different cultivars. For example, susceptible and resistant potato and alfalfa cultivars show the same pattern of variability of nematodes that penetrated the roots at early time points of infection [9,32]. In addition, wheat susceptible and resistant cultivars do not reveal differences in penetration rates of *P. thornei* [48], while sugarcane resistant and susceptible clones show no significant differences in the penetration at 12 and 24 h after inoculation with *P. zeae* [49].

The interaction of *P. penetrans* can trigger diverse reactions of the roots dependently of the host plant, namely, the formation of minute to large necrotic lesions associated with nematode feeding of and migration in the epidermal and cortex cells of the roots [1,5,6]. Root phenotypic reactions induced upon RLN infection are associated with a complex activation of different host genes, such as cell-wall-related genes, the phenylpropanoid pathway, and defense signaling pathways, among others [9,50,51]. In our study, cultivars Stemster, Royata, and Laura exhibited the strongest reaction upon early nematode infection,

as early as 1 DAI. Interestingly, the last two shared some commonalities in their genetic background (Table S1). Host differences in lesion development have been not only reported in *P. penetrans* [6,52–54] but also in other species of this genus [48,55,56]. These host-specific root cellular reactions may be a consequence of the host defense mechanism activation, which could impair nematode development and/or suppression of nematode reproduction [9,50,51,57] but may also promote egression of nematodes into the soil [32,49]. For example, the egression of nematodes from the roots was previously suggested as related to a potential resistance mechanism of some potato cultivars to *P. penetrans* [33–35]. A large percentage of nematodes egressed from cultivar Laura (~57%), in comparison to the other cultivars (~30–44%), resulting in a lower number of nematodes effectively established within the roots of this cultivar at 15 DAI. Previous studies also reported differences in the rate of nematode egression between females and males according to the susceptibility of the potato cultivars. France and Brodie [34,35] suggest that females egress earlier than males or juveniles in potato clones considered to have some level of resistance to *P. penetrans*, implying that females are more sensitive to host susceptibility. In our study no differences among cultivars were detected in the ratios of egressed females and males. Nevertheless, following the higher egression of nematodes from cultivar Laura, a significant reduction in the number of deposited eggs at 15 DAI was observed for this cultivar, potentially related to the higher rate of egressed nematodes or some type of inhibition on the reproduction performance of *P. penetrans* of this cultivar.

Our findings highlight that commercial potato cultivars possess a diverse range of ability to support the reproduction of *P. penetrans*, although nematode multiplication was not prevented in any of the cultivars. Quantification of nematodes from roots proved to be difficult and time consuming, as few nematodes egressed from roots into water after 7 days and most nematodes had to be counted by dissection of stained roots. Nematode extraction techniques using longer periods of root incubation in water together with root maceration techniques should be considered in future studies focusing on RLN root quantification. Cultivars Agria, Stemster, and Kennebec are among the cultivars that supported larger numbers of nematodes at 60 DAI, while the total number of nematodes recovered from a gram of root in cultivar Laura was significantly reduced in comparison to the other cultivars, following the same pattern of results obtained at the early time points. The lower numbers of nematodes found in cultivar Laura may be correlated to the higher number of initially egressed nematodes, as a consequence of the strong reaction observed in the roots, consequently leading to a reduction in the number of deposited eggs as well. Although Brodie and Plaisted [32] found that some potato clones resistant to *Globodera* spp. were also resistant to *P. penetrans*, in our study, cultivars reported to hold resistance to *G. pallida* and *G. rostochiensis*, such Camel and Dirosso, supported the multiplication of the *P. penetrans* isolate used in this study. Nevertheless, the lack of a complete resistance response/non-reproduction examined has the same patterns observed previously for other potato cultivars [28,31,32,34,35]. Even though using cultivars that can restrict nematode development and reproduction may reduce the nematode impact on potato yield, and thus can offer an acceptable alternative to help diminish nematode impact, their use should be combined with other management strategies [17]. On the other hand, most of the cultivars tested here showed a high rate of infection based on the SI using the cultivar Kennebec as a known susceptible host. The fact that most of these cultivars are commonly used in potato fields in Europe could not only exponentiate the transmission of nematodes into non-nematode-infested fields, but also allow the maintenance and increase of the initial populations present in those fields. For example, most of the potato fields surveyed in Portugal revealed the presence of *P. penetrans* and other RLN species in association with cultivars such as Agria, Astérix, Kennebec, and Picasso [20].

In this study, no differences in the numbers and weight of tubers were found among the cultivars of inoculated versus non-inoculated plants; however, tuber growth was restricted and limited to the pot size and different results may be achieved under field conditions. Apart from the susceptibility of the cultivar, damage caused by *P. penetrans* to

potato can also vary according to the initial nematode population densities and the edaphic conditions [1,28,31]. Using an initial nematode population density of four nematodes g/soil, the variability in the number of nematodes reproducing in the ten cultivars generally agree with the high variability found for other cultivars reported in the literature [28,31,32]. Although our data suggest a large range of susceptibility among the cultivars, field assays are needed to re-assess the impact of *P. penetrans* on the effective yield production. Further studies on histopathology and the molecular mechanisms behind the interaction of *P. penetrans* and potato plants are still poorly understood and should be analyzed. To conclude, our findings reveal that commercial potato have different susceptibility to *P. penetrans*, reinforcing the importance of cultivar selection in RLN management.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8030244/s1, Figure S1: Percentage of *Pratylenchus penetrans* stages (adults, juveniles and eggs) inside potato roots of cultivars Agria, Camel, Royata, Laura, Kennebec, and Stemster at 1,3,7 and 15 days after inoculation (DAI) with 750 *P. penetrans* mixed stages/plant; Figure S2: Ratio of female/ male nematodes egressed throughout six days from potato cultivars, Kennnebec, Camel, Laura, Royata, Stemster and Agria, 3 days after inoculation with 1000 *Pratylenchus penetrans*; Figure S3: Plant phenotype (aerial part) of non-inoculated (control) versus nematode-infected potato cultivars at 60 days after inoculation in assay 1, where the following cultivars were screened: (A) Agria, (B) Désireé, (C) Kennebec, (D) Laura, (E) Picasso and (F) Stemster. Inoculations were performed with 8000 mixed stages of *Pratylenchus penetrans*/plant.; Figure S4: Plant phenotype (aerial part) of non-inoculated (control) versus nematode-infected potato cultivars at 60 days after inoculation in assay 2, where the following cultivars were screened: (A) Agata, (B) Camel, (C) Dirosso, (D) Kennebec, (E) Laura and (F) Royata. Inoculations were performed with 8000 mixed stages of *Pratylenchus penetrans*/plant.; Table S1: Parents of potato cultivars used in this work, obtained from Potato pedigree database: https://www.plantbreeding.wur.nl/PotatoPedigree/index.html, accessed on 4 February 2022. *Globodera* spp. resistance information for potato cultivars. Pa *Globodera pallida* and Ro *Globodera rostochiensis*.

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