Resistance of carrot cultivars to *Meloidogyne chitwoodi*.

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**Abstract.** Carrot (*Daucus carota*) is a very important vegetable for both the processing industry and fresh market. It is cultivated worldwide. However, carrot cultivation is hampered by diseases caused by organisms such as plant-parasitic nematodes like *Meloidogyne chitwoodi*. This quarantine pest has been reported to cause serious damage to carrot yields in areas of carrot production. Different control strategies for *M. chitwoodi* exist but the most desirable is to sort for resistant and tolerant cultivars. To do this, six commercial carrot cultivars (*ABK, Berlanda, Mokum, Nefac, Parmex* and *Sweetheart*) which are cultivated in Belgium were screened for their resistance to this pest. Therefore, nematode penetration, development and reproduction were observed. Plants of each cultivar were grown in a climate controlled chamber, inoculated with same initial densities of juveniles of *M. chitwoodi*. Penetration and development were examined 2, 4, 6, 12, 24 and 48 days after inoculation. Sixty days after inoculation, the presence of egg masses was assessed. For all cultivars, penetration was evident from day 2 though with subsequent observations, there was a variation between the cultivars for penetration and development of the inoculated J2. After 60 days, egg masses were found on all the cultivars. However, on cv. *Parmex* only 10% of the tested plants contained egg masses. For cvs *Berlanda* and *Nefac*, this was 60% and on cv. *Sweetheart*, 100% of the tested plants contained egg masses. From the hatching experiment set up over a period of 8 weeks, the percentage hatch was similar between the cultivars but the number of eggs per egg mass was smaller for cvs *Parmex* and *Berlanda*. This is indicative that there could be some potential of resistance in these cultivars compared to the other cultivars.
Further screening with different populations of *M. chitwoodi* from different origin is required to evaluate the resistance status of these two cultivars.

**Keywords:** Penetration, development, resistance screening, hatching.
INTRODUCTION

Carrot, *Daucus carota* is a very important vegetable both for the processing industry and for fresh market. It is cultivated throughout the world especially in the tropics and temperate areas, being the second most popular vegetable after potatoes (Anonymous, 2008a). The cultivated type of carrot is called *Daucus carota* ssp *sativus* which belongs to the Apiaceae (Umbelliferae) and has a number of wild relatives such as *D. carota* ssp *drepanensis*, *D. carota* ssp *gummifer*, *D. carota* ssp *maximus*, *D. carota* atrorubens and *D. muricatus* (Heywood 1983).

Production of carrot worldwide has faced a significant constraint due to plant-parasitic nematodes of which more than 90 species across different genera been associated to it including *Pratylenchus*, *Meloidogyne*, *Longidorus*, *Paratylenchus*, *Paratrichodorus*, *Belonolaimus*, *Rotylenchus* and *Ditylenchus* (Davis & Raid, 2002). The ‘cosmetic injury’ caused as a result of forking and galling symptoms on the marketable taproot is responsible for significant yield losses observed in carrot cultivation (Roberts, 1988). *Pratylenchus* species have been reported to cause forking and death of carrot (Coosemans, 1975) and also reduction in size and branching of the taproot (Vrain & Belair, 1981).

There exist different management options for the control of plant parasitic nematodes and these strategies include biological control (Sharma & Pandey, 2009; Vagelas et al., 2007), chemical control, (Giannakou et al., 2004), crop rotation systems (Kratovil et al., 2004), use of solarisation, weed management, use of green manure (Bar-Eyal et al., 2006), influence on date of planting and harvesting (Roberts, 1987) but the most sustainable is the use of resistant and tolerant cultivars.

Resistance to *M. chitwoodi* has been reported in the potato species *Solanum bulbocastanum*, *S. hougasii* (Brown et al., 2006), and *S. fendleri* (Janssen et al., 1996). There has been reported resistance to some root-knot nematodes such as *M. javanica* from several carrot cultivars.
including ‘Brasilia’ (Huang 1986; Huang et al., 1986) but the resistance and tolerance of carrot to \textit{M. chitwoodi} as well as the effect of \textit{M. chitwoodi} on carrots has little information (Wesemael & Moens, 2008a). Carrot cultivars were classified as non hosts, moderate hosts or good hosts depending on the multiplication of different races of \textit{M. chitwoodi} (Mojtahedi \textit{et al.}, 1988; Santo \textit{et al.}, 1988). In the canning industry, carrot uniformity and quality of its taproot is of more importance than just the maximum yield. This is because not only forked or stubby roots but also taproots containing heavy galls cannot be processed. As a result of the high sensitivity of the carrot taproot to the damaging effects of nematode infection and also due to the undesirability for the continuous use of nematicides, developing carrot cultivars resistant and tolerant to root-knot nematodes is of paramount importance.

This work was based on screening different commercial carrot cultivars for resistance to \textit{M. chitwoodi} with the objectives being:

- Screen which cultivars were resistant to \textit{M. chitwoodi} based on comparison of multiplication rate and number of egg masses of \textit{M. chitwoodi} on the different cultivars.

- Investigate the mechanism behind any possible resistance found.
MATERIALS AND METHODS.

PREPARATION OF *MELOIDOGYNE CHITWOODI* CULTURE.

Plants of tomato, *Lycopersicon esculentum* cv. Moneymaker were used to maintain a stock culture of *M. chitwoodi*. Sowing of the tomato seeds was done in October, 2009. After germination, the tomato seedlings were transplanted in plastic pots (diameter 17cm) filled with sterilized sandy soil and growth was carried out in a temperature-controlled glasshouse (20-26°C) with 14 hours light daytime duration. Sterilized soil (18 hours at 100°C) was used in order to prevent pre-infection of the tomato plants with plant-parasitic nematodes prior to nematode inoculation while soil type was preferred as sandy to reflect the growth conditions in the field.

One month after the tomato plants were transplanted to the plastic pots, they were inoculated with second stage juveniles (J2) of *M. chitwoodi*. The J2 of *M. chitwoodi* used for inoculation of the tomato plants were obtained from a naturally infested field in the province of Antwerp, Belgium were previous field work on the effects of *M. chitwoodi* on carrots has been done.

EXTRACTION OF *M. CHITWOODI* INOCULUM FROM TOMATO PLANTS.

Three months after inoculation of the tomato plants with *M. chitwoodi* J2, the plants were harvested from the greenhouse. The roots of the tomato plants were carefully removed from the soil, washed and cut into pieces of 1-2cm and placed on the modified Baermann’s funnel (Hooper, 1986) in order to obtain freshly hatched J2. This was done 1-2 days prior to inoculation of the carrot cultivars.
CARROT CULTIVATION AND SCREENING FOR RESISTANCE.

In order to evaluate the host resistance of carrot cultivars to *M. chitwoodi*, 6 different carrot cultivars were used, *ABK, Berlanda, Mokum, Nefac, Parmex* and *Sweetheart*, 160 plants per cultivar. These seeds originated from Bejo, a company specialized in producing vegetable seeds based in the Netherlands. The growth period of these cultivars ranges between 90 to 130 days after sowing with some maturing earlier than others. The treatments consist of the following:

- Screening for penetration rates, and developmental stages of *M. chitwoodi* in the different cultivars after different time intervals (observation); 2, 4, 6, 12, 24 and 48 days after inoculation and 10 replicates per cultivar were used for each observation.

- Screening for formation of egg masses and hatching experiment: Ten plants per cultivar were screened 8 weeks after inoculation. Egg masses from the replicates per cultivar where egg masses were formed, were used to set up a hatching experiment.

Plants were grown in plastic folding tubes of dimensions 15× 20 × 120 mm, in a climate chamber of average daytime temperature 22.82± 0.129°C and average night temperature of 19.45± 0.507°C. Plastic tubes were filled with sterilized sandy soil of pH 5.8 and percentage organic content 2.54%, with one seed sown per tube. Sowing was done on the 4th of February, 2010. Watering of plants was done by using an atomizer. Twenty days after planting, the seeds germinated to young plants which were carefully inoculated with 200 freshly hatched J2 (less than 24 hours-old) using a micropipette. Screening was done subsequently for 2, 4, 6, 12, 24, 48 days after inoculation and after 60 days for formation of egg masses.
Screening for penetration rates and developmental stages:

After inoculation of each plant with 200 J2, screening for penetration rate and developmental stages was done. To remove the roots of the plants from the tubes, the tubes were placed in a water bath and the soil was carefully removed from the roots. The methodology for staining roots was based on the technique of Byrd et al. (1983). For each observation, the roots of the replicates were washed with tap water, placed in a 150ml beaker and cut into pieces of 1-2cm. Fifty ml of tap water and 20ml of chlorine bleach (5.75% NaOCl) were added to clear the roots. These roots were soaked for 4 minutes in this NaOCl solution while being agitated occasionally. The roots were then washed with tap water on sieves to remove excess bleach.

The water was then drained and the roots transferred to a glass beaker containing 30ml of tap water. One ml of stock acid-fuchsia stain solution (3.5 g acid fuchsia in 250ml acetic acid and 750ml distilled water) was added to the water and boiled for 30 seconds on a hotplate. The solution was then cooled to room temperature, drained and the roots rinsed in running tap water. The roots were boiled in 20-30ml of glycerin, distributed on a Petri-dish and observed with the aid of a binocular microscope.

To check for the presence of males, the soil from the plants which were screened 48 days after inoculation were used. The soil of each replicate was collected separately in1l beakers, filled with tap water and centrifuged with the Hendrickx automated zonal centrifugal machine (Hendrickx, 1995) which is a machine based on the principles of conventional centrifugation. After this extraction, the nematodes were collected in glass beakers while the sandy fraction was discarded.
Screening for formation of egg mass and hatching experiment.

Sixty days after inoculation, 10 plants per cultivar were harvested and screened for the number of egg masses formed. To do this, the technique of Daykin & Hussey (1985) was used. Phloxine B (0.15-0.20g) was dissolved in a beaker filled with 1000ml of tap water. The root systems of the plants were then carefully washed with tap water and then soaked into the Phloxine B solution. The roots were left for 15-20 minutes in the solution which stained the gelatinous matrix of the egg masses. Later, the roots were removed from the solution, washed with tap water to remove residual stain and then observed with the aid of a binocular microscope for egg masses. In addition to counting the number of egg masses, the hatching process was examined. Three egg masses per infected plant were collected except for cv. Parmex which had just one infected plant with one egg mass. The egg masses were placed in plastic tubes containing a 48µm sieve that retained the egg masses but allowed the hatched J2 to go through. Distilled water was added to these plastic tubes and kept in an incubator at mean daily temperature of 19.71± 0.026 °C. Counting of the number of J2 that hatched out per replicate was done weekly over a period of 8 weeks and the distilled water was refreshed on a weekly basis.

STATISTICAL ANALYSIS.

Statistica 9 was used for the analysis of the data obtained. Transformation was done for some data in order to meet the requirements of Analysis of Variance (ANOVA). For data which could not meet the requirements of ANOVA, Kruskal Wallis ANOVA test was used. For analysis of the hatching process, a logistic model was used to fit the hatching data obtained; 
\[ y = c / (1 + \exp (-b*(time-m))) \]
where y= the cumulative percentage hatch, c= the final hatching percentage, b= the hatching rate and m= time at which 50% hatch was attained. These results were calculated for all the replicates of the cultivars separately. For data
analysed using ANOVA, the results were reported as either significant or non significant using the Turkey honest significant difference test (p< 0.05) while for those analysed with Kruskal Wallis ANOVA test, the results were reported as either significant or non significant using the Non-parametric pair wise test. Paired sample T test was used to analyze the results of penetration and developmental stages within each cultivar per time period (subsequent observation period). The data for filiform juveniles of day 12 was transformed using the Square root function while for day 24; the data for filiform and swollen juveniles were transformed using the LOG10 function. For day 48, the data for filiform and swollen juveniles were transformed by Square root and LOG10 functions respectively while non-parametric analysis was used for the data of males and females. The data of the mean number of eggs per egg mass were transformed using the Square root function.
RESULTS.
Screening for Penetration and Developmental Stages.

From day 2 to day 48, J2 penetration and development were observable (Figure 1). Two days after inoculation, only filiform stage was present in the roots of the tested plants with the highest mean numbers of penetrated J2 found in cvs Parmex and Berlinda. There was no significant difference (p>0.05) between the cultivars (Table 1). Four days after inoculation, only the filiform stage was still present though penetration numbers were higher than for day 2 (Table 1). The highest mean penetrations were recorded in cvs Parmex, Nefac and ABK but there was no significant difference between the cultivars (p>0.05). At 6 days after inoculation, no other stages of M. chitwoodi had been formed except the filiform stage. However, there was a significant difference (p<0.05) in the number of J2 penetration per cultivar (Table 1). From day 12 to day 48, all developmental stages were found inside the roots. After 12 days, both filiform and swollen juveniles were found (Table 2). From day 24, filiform juveniles, swollen juveniles and females could be differentiated (Table 2) while after 48 days, filiform juveniles; swollen juveniles, females and males could be differentiated (Table 3). At day 12, cvs Berlinda, Nefac, Parmex and Sweetheart recorded more filiform J2 than swollen J2 while cvs Mokum and ABK recorded more swollen J2 than filiform juveniles. Statistical analysis of the data for filiform juveniles and data for swollen juveniles show there was significant difference between the cultivars (p<0.05). At day 24, there was no significant difference between cultivars (p>0.05) for filiform juveniles and swollen juveniles. There was a significant difference between the cultivars on the number of females formed (p<0.05). Cv Sweetheart was significantly different from cvs Berlinda, Mokum, Nefac and Parmex. Day 48 results showed there was a significant difference (p<0.05) between the cultivars for the different stages of nematode present except for the males where there was no significant difference (p>0.05). For the filiform juveniles, cv. Sweetheart was significantly different from
cv. Mokum, for swollen juveniles, cvs ABK and Sweetheart were significantly different from cv. Parmex, in the females, cvs Parmex and Berlanda were significantly different from cvs Mokum and Sweetheart, while cv. Mokum was significantly different from cvs Nefac and Sweetheart. The significance of penetration and development of the penetrated J2 against observation time showed there was a significant difference in observation time per cultivar (Table 4). In cv. Sweetheart, day 2 was significantly different from day 4, in cv. Mokum, there were significant differences between day 2 and 4, day 4 and 6. In cv. ABK, there was significant difference between days 2 and 4, days 4 and 6. In cv. Parmex, there was significant difference between days 2 and 4, days 4 and 6, days 6 and 12, days 24 and 48. In cvs Berlanda and Nefac, there was significant difference between days 2 and 4, days 4 and 6, days 24 and 48.

Figure. 1. Mean number of Meloidogyne chitwoodi present per cultivar after Days 2, 4, 6, 12, 24 and 48.

Mean number of M. chitwoodi present per cultivar after Days 2, 4, 6, 12, 24 and 48.

Figure. 1. Mean number of Meloidogyne chitwoodi present 2, 4, 6, 12, 24 and 48 days after inoculation of six carrot cultivars.
Table 1. *Mean number (±SE) of second-stage juveniles of Meloidogyne chitwoodi found in the roots of six carrot cultivars 2, 4 and 6 days after inoculation. Different letters per column indicate significant differences (p<0.05)*

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>day 2</th>
<th>day 4</th>
<th>day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetheart</td>
<td>0.30±0.30</td>
<td>27.90±4.72</td>
<td>39.80±10.43a</td>
</tr>
<tr>
<td>Mokum</td>
<td>3.20±1.55</td>
<td>22.60±5.77</td>
<td>9.40±3.70b</td>
</tr>
<tr>
<td>ABK</td>
<td>3.00±1.28</td>
<td>30.80±7.25</td>
<td>24.90±5.02ab</td>
</tr>
<tr>
<td>Parmex</td>
<td>8.30±2.71</td>
<td>35.10±5.80</td>
<td>41.40±5.39a</td>
</tr>
<tr>
<td>Berlanda</td>
<td>5.50±2.92</td>
<td>25.60±4.27</td>
<td>14.10±4.87b</td>
</tr>
<tr>
<td>Nefac</td>
<td>0.5±0.50</td>
<td>34.80±6.74</td>
<td>42.70±5.09a</td>
</tr>
</tbody>
</table>

Table 2. *Mean number (±SE) of developmental stages of Meloidogyne chitwoodi found in the roots of six carrot cultivars 12 and 24 days after inoculation. Different letters per column indicate significant differences (p<0.05) (fil.juv=filiform juveniles, Swol.juv=swollen juveniles)*

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>day 12</th>
<th>day 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetheart</td>
<td>9.70±1.94a</td>
<td>6.40±1.55a</td>
</tr>
<tr>
<td>Mokum</td>
<td>5.70±1.32ab</td>
<td>14.60±2.29ab</td>
</tr>
<tr>
<td>ABK</td>
<td>11.20±2.44a</td>
<td>17.20±2.67b</td>
</tr>
<tr>
<td>Parmex</td>
<td>12.70±3.81a</td>
<td>5.30±1.92a</td>
</tr>
<tr>
<td>Berlanda</td>
<td>18.50±2.11ac</td>
<td>11.00±3.50ab</td>
</tr>
<tr>
<td>Nefac</td>
<td>23.60±2.60bc</td>
<td>7.20±2.09ab</td>
</tr>
</tbody>
</table>
Resistance of carrot cultivars to Meloidogyne chitwoodi

Table 3. Mean number (±SE) of developmental stages of Meloidogyne chitwoodi found in the roots of six carrot cultivars 48 days after inoculation. Different letters per column indicate significant differences (p<0.05)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>fil.juv ±SE</th>
<th>Swol.juv ±SE</th>
<th>females ±SE</th>
<th>Males ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetheart</td>
<td>4.70±1.57a</td>
<td>10.50±1.83a</td>
<td>2.90±0.87a</td>
<td>0.50±0.22</td>
</tr>
<tr>
<td>Mokum</td>
<td>0.70±0.26b</td>
<td>4.80±0.84ab</td>
<td>7.20±1.66b</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>ABK</td>
<td>3.00±0.91ab</td>
<td>10.70±2.67a</td>
<td>3.20±1.22ab</td>
<td>0.20±0.13</td>
</tr>
<tr>
<td>Parmex</td>
<td>4.60±1.62ab</td>
<td>1.70±0.45b</td>
<td>0.30±0.21c</td>
<td>0.70±0.37</td>
</tr>
<tr>
<td>Berlanda</td>
<td>2.40±0.62ab</td>
<td>5.90±1.77ab</td>
<td>0.70±0.70c</td>
<td>1.40±0.37</td>
</tr>
<tr>
<td>Nefac</td>
<td>1.20±0.33ab</td>
<td>6.60±1.64ab</td>
<td>3.10±1.50ac</td>
<td>0.20±0.13</td>
</tr>
</tbody>
</table>

Table 4. Mean number (±SE) of Meloidogyne chitwoodi found in the roots per cultivar between the days after inoculation. Different letters per column indicate significant differences (p<0.05).

<table>
<thead>
<tr>
<th>Time</th>
<th>sweetheart</th>
<th>Mokum</th>
<th>ABK</th>
<th>Parmex</th>
<th>Berlanda</th>
<th>Nefac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>0.30±0.30a</td>
<td>3.20±1.55a</td>
<td>3.00±1.28a</td>
<td>8.30±2.71a</td>
<td>5.50±2.92a</td>
<td>0.50±0.50a</td>
</tr>
<tr>
<td>Day 4</td>
<td>27.90±4.72b</td>
<td>22.60±5.77b</td>
<td>30.80±7.25b</td>
<td>35.10±5.80b</td>
<td>25.60±4.27b</td>
<td>34.80±6.74b</td>
</tr>
<tr>
<td>Day 6</td>
<td>39.80±10.43b</td>
<td>9.40±3.70c</td>
<td>24.90±5.02c</td>
<td>41.40±5.39c</td>
<td>14.10±4.87c</td>
<td>42.70±5.09c</td>
</tr>
<tr>
<td>Day 12</td>
<td>16.10±2.98b</td>
<td>20.30±2.33c</td>
<td>28.40±4.03c</td>
<td>18.00±4.84d</td>
<td>29.50±4.31c</td>
<td>30.80±2.64c</td>
</tr>
<tr>
<td>Day 24</td>
<td>21.90±2.80b</td>
<td>20.00±3.26c</td>
<td>19.40±2.58c</td>
<td>19.00±2.54d</td>
<td>25.10±2.33c</td>
<td>22.00±3.00c</td>
</tr>
<tr>
<td>Day 48</td>
<td>18.60±2.82b</td>
<td>12.70±1.79c</td>
<td>17.00±3.45c</td>
<td>7.00±1.89e</td>
<td>9.20±2.36d</td>
<td>11.00±2.56d</td>
</tr>
</tbody>
</table>

Egg masses were found on all cultivars with the highest mean numbers found in cvs. Mokum, Sweetheart and ABK while cv. Parmex had the least mean number of egg masses. Non-parametric analysis of the data showed there was a significant difference (p<0.05) between the cultivars on the number of egg masses formed (Figure 2). There was significant difference between the mean numbers of eggs per egg mass between the cultivars (Figure 3). However,
there was no significant difference on the cumulative percentage hatch of J2 between the cultivars (Table 6). The hatching curves per cultivar are shown in figure 4.

Figure 2. Mean number of egg masses per tested plant of Meloidogyne chitwoodi found in the roots of six carrot cultivars 48 days after inoculation. Different letters on bars indicate significant differences (p<0.05)

Table 5 Percentage of plant with egg masses and mean number (±SE) of egg mass per infected plant of six carrot cultivar.

<table>
<thead>
<tr>
<th>cultivar</th>
<th>sweetheart</th>
<th>Mokum</th>
<th>ABK</th>
<th>Parmex</th>
<th>Berlanda</th>
<th>Nefac</th>
</tr>
</thead>
<tbody>
<tr>
<td>%plant with egg mass</td>
<td>100%</td>
<td>80%</td>
<td>70%</td>
<td>10%</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>Mean egg mass/infected plant</td>
<td>7.40±1.14</td>
<td>9.25±1.86</td>
<td>8.86±2.19</td>
<td>1.00±0.32</td>
<td>5.50±1.86</td>
<td>3.17±1.06</td>
</tr>
</tbody>
</table>
Figure 3. *Mean number of eggs per egg mass* of *Meloidogyne chitwoodi* of six carrot cultivars. Different letters on bars indicate significant differences (p < 0.05).

Figure 4. *Hatching process of six carrot cultivars* obtained after 8 weeks.
Table 6. Mean number (±SE) of the hatching parameters c, b and m for the different cultivars. There was no significant difference in these mean values between the cultivars (p>0.05).

<table>
<thead>
<tr>
<th>cultivar</th>
<th>c</th>
<th>b</th>
<th>m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetheart</td>
<td>99.12±1.58</td>
<td>1.25±0.10</td>
<td>2.22±0.07</td>
</tr>
<tr>
<td>Mokum</td>
<td>98.83±1.49</td>
<td>1.22±0.09</td>
<td>2.15±0.07</td>
</tr>
<tr>
<td>ABK</td>
<td>97.36±1.59</td>
<td>1.30±0.11</td>
<td>2.08±0.08</td>
</tr>
<tr>
<td>Parmex</td>
<td>99.09±1.92</td>
<td>1.63±0.19</td>
<td>2.06±0.08</td>
</tr>
<tr>
<td>Berlana</td>
<td>96.59±1.69</td>
<td>1.60±0.17</td>
<td>1.83±0.08</td>
</tr>
<tr>
<td>Nefac</td>
<td>97.70±1.43</td>
<td>1.71±0.16</td>
<td>2.11±0.06</td>
</tr>
</tbody>
</table>
DISCUSSION.

Carrots have been described as good host for *Meloidogyne chitwoodi* (Wesemael & Moens, 2008a). However, the results obtained from this experiment show that penetration and reproduction of *M. chitwoodi* vary between different carrot cultivars. For some cultivars, penetration was faster but reproduction slower whereas in others, penetration started at a slower rate but reproduction became very rapid. Cultivars with the least mean number of egg masses were *cvs* Parmex Nefac and Berlanda. These results were similar to earlier findings by Wesemael and Moens(2008a) where for *cvs. Parmex* and Berlanda, there were no egg masses on more than 80% of the tested plants. However, there was a difference in the mean number of egg masses found on *cvs. Berlanda* and Parmex from this result and their earlier finding. From this work, *cv. Parmex* had fewer egg masses than *cv. Berlanda* while the previous finding showed *cv. Berlanda* had fewer egg masses than *cv. Parmex*. Nonetheless, compared to the other cultivars, these two cultivars had relatively fewer egg masses. This suggests that these cultivars may have some potential for resistance.

*Meloidogyne chitwoodi* juveniles do penetrate the roots directly behind the root cap although this could also be through; spots where lateral roots emerge, at penetration sites of other juveniles and wounded surfaces of roots (Hussey, 1985). Their penetration, activity and reproduction are at best at optimum temperatures. Santo and O’Bannon (1981) described that *M. chitwoodi* has a higher reproductive potential at lower temperatures ranging between 15°C, 20° and 25°C but not 30°C. Thus the mean daytime temperature (22.82± 0.129°C), mean night temperature (19.45± 0. 507°C) in the climate chamber and the mean temperature (19.71±0. 026 °C) in the incubator were adequate enough to enhance the activity of *M. chitwoodi*.

When J2 of *M. chitwoodi* penetrate a root tip, they form a feeding site (Goverse *et al*., 2000). However, penetration and development of J2 depend on both pre-infectional and post-
infectional defence mechanisms in the plants (Huang, 1985; Paxton, 1975, Veech, 1982).

Over the period of observation from day 2 to day 48, the mean numbers of *M. chitwoodi* present had an almost similar trend for most cultivars with least numbers of penetrated J2 observed at day 2. The tendency of *M. chitwoodi* penetration and development in the different carrot cultivars was different with *cvs Berlanda* and *Parmex* exhibiting some degree of resistance compared to the other cultivars.

In *cv. Parmex*, during the early stages from day 2, there was high population build-up till day 6 compared to the rate in the other cultivars. This might have been as a result of its poor pre-infectional defence mechanisms and also its differential root system. Balhadere and Evans (1972) described that the relationship between root size and the number of nematodes is dynamic and ignoring root size at inoculation may potentially ignore the mechanisms by which nematode invasion, establishment and development may be affected given their observations on the direct and indirect mechanisms observed with *M. naasi*. Also these weak pre-infectional defences could be as a result of physically penetrable roots since a possible resistant mechanism could be attained by impenetrable roots (Dropkin & Nelson, 1960).

However, the reduction in the number of penetrated J2 after day 6, higher mean numbers of filiform juveniles than swollen juveniles (day 12), higher mean numbers of filiform juveniles than swollen juveniles and females (day 24 and day 48) compared to the other cultivars indicate a reduction or delay in the development of *M. chitwoodi*. This might be a plant response to infection resulting in the production of plant chemicals or hormones such as phytoalexins which altered the metabolic pathways of these nematodes (Huang, 1985). It could also be explained that the *cv. Parmex* after penetration by the J2, altered the metabolites required for nematode nutrition, leading to deficiencies which intend caused the penetrated J2 to emigrate from the roots or decreased their reproductive capacity for those that remained in the roots (Giebal, 1974). The high male to female ratio in the *cv. Parmex* indicates it might
have some resistance properties. Completely developed males have been found in roots of
resistant plants (Berge et al., 1974). The presence of more males suggests the conditions in
the roots were not favorable for many females to develop so more males were formed which
left the roots. The presence of fewer females could explain why cv. Parmex recorded the least
mean number of egg masses. The delay in the developmental stages in cv. Parmex suggest M.
chitwoodi might have a prolonged life cycle in this cultivar while the presence of fewer
swollen juveniles and females compared to the other cultivars indicate some degree of
resistance given that more rapid maturation of females and greater numbers of females per
plant could be used to distinguish resistant plants from susceptible ones (Cook et al., 1999).
Despite showing no hypersensitive reactions such as necrosis, the potential resistance in cv.
Parmex seems different; several days after penetration, many J2 were still present coupled
with males formed. Given that resistance is related to retarded nematode penetration,
development, egg production and fast plant growth which results in a low nematode
population density with few nematodes completing their life cycle (Huang, 1986), cv. Parmex
possesses these qualities and thus could have some potential for resistance.

Cv. Berlanda similar to cv. Parmex had higher mean numbers of penetration compared to the
other cultivars except for day 4. This fluctuation in the mean numbers of penetrated J2 might
be as a result that resistance genes do exhibit different defence mechanisms upon infection.
Fuller et al. (2008) reported that host plants which have resistance genes do possess different
defence mechanisms for their defence against plant parasitic nematodes. The high build-up on
day 24 compared to the other cultivars might be due to breakdown in pre-infectional defence.
Some populations of M. chitwoodi have shown variability in virulence leading to suppression
of plant defence mechanisms (Van der Beek et al., 1998). However, the presence of fewer
swollen juveniles and females compared to filiform juveniles indicate that the plant might
have mounted post-infectional defence mechanisms involving chemical reactions in the root
to alter biochemical and physiological processes (Huang, 1985). Also, the absence of females after day 24 compared to the other cultivars and presence of males after day 48 is indicative of some potential of resistance.

*Cv. Nefac*, based on penetration had the most steady rate of increase in penetration from day 2 to day 24 compared to the other cultivars. This might be as a result of poor or weak basal plant defences and greater nematode-root encounter given the size of its root system. Despite the increase in the mean number of penetrated J2 and development to other stages, there was the formation of few egg masses on the plants of this cultivar. This suggests *cv. Nefac* might have a potential to retard the female from producing numerous egg masses.

In *cv. ABK*, there was even a more fluctuation in the mean numbers of penetrated J2 throughout the observation days but despite the number of J2 to have penetrated, there was a faster development to other stages (swollen juveniles and females) than for most cultivars. This could be observed for days 12 to 48 where it had the highest mean number of swollen J2 (day 12 and day 48), and second in mean numbers of females for both days 24 and 48. Thus the fluctuation in the number of penetrated J2 might be explained either by the fact that nematode-root encounter was dynamic or the plant tried to mount some temporal defence to prevent J2 entry. Nonetheless, the presence of more developmental stages despite the smaller number of nematodes that penetrated compared to other cultivars suggest *M. chitwoodi* could reproduce so well on *cv. ABK* and have a shorter life cycle. Given that *cv. ABK* was among the first 2 cultivars to exhibit developmental stages at early observations suggest the J2 which penetrated could have easily established feeding sites.

*Cv. Mokum* was very alike to *cv. ABK*. It was at the bottom of the cultivars with least mean number of penetrated J2 except for days 2 and 48. But the striking fact was that despite the small number of J2 that penetrated compared to the other cultivars, they had a more rapid
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change to swollen juveniles (day 12 and 24) and females (day 48). The fact that there were no males found in cv. Mokum also suggests this cultivar is a very good host for M. chitwoodi. The life cycle of M. chitwoodi could as well be very short in this cultivar given that it recorded the highest mean number of females after 48 days moving from 3rd highest after day 24.

In cv. Sweetheart, penetration and development was similar as for cvs. ABK and Mokum. After day 24, it recorded the highest mean number of females compared to the other cultivars suggesting M. chitwoodi had the shortest life cycle in this cultivar. All the tested plants of this cultivar had egg masses implying reproduction was high. However, males were also found in this cultivar which was indicative of some resistance. This might have been as a result of some localized cell resistance to the J2, induced by the mounting of a hypersensitive response resulting to the formation of necrotic cells which prevented the establishment of feeding sites (Paulson & Webster, 1972; Dropkin, 1969).

The hatching results also showed that cvs Sweetheart, ABK and Mokum had more eggs per egg mass indicating reproduction in these cultivars was better than for the other cultivars. Despite the fact that the percentage hatch per cultivar was not different indicating M. chitwoodi has almost the same rate of hatching irrespective of the cultivar, the nematode density of J2 hatching in the soil per cultivar will be different regarding the differences in size of egg masses per cultivar.

Previous results of Wesemael and Moens (2008a) showed that cvs. Mokum, Sweetheart and ABK had the highest mean numbers of egg masses among the tested cultivars which is similar to these results. However, while in their findings cv. ABK had the highest mean number of egg masses followed by cvs Sweetheart and Mokum, these results show that cv. Mokum had the highest mean egg mass followed by cvs Sweetheart and ABK but this was not significant.
Early sowing of seeds when temperatures are low in *M. chitwoodi* infested fields could be done given that J2 decline in numbers during winter (Pinkerton et al., 1991). This will enable limited reproduction on the carrot cultivars and by the time infective J2 populations start increasing due to slight increase in soil temperature and favorable hatching from eggs (Wesemael & Moens, 2008b; Pinkerton et al., 1991); the plants must have grown rendering penetration by J2 very difficult. In situations of choice for cultivation during temperatures which favour *M. chitwoodi* reproduction, cultivars which restrict reproduction are advisable to be cultivated.

From these results, it could be inferred that there is some potential for resistance in the commercial carrot cultivars *Berlanda* and *Parmex*. However, different populations of *M. chitwoodi* have been observed to reproduce differently on carrots (Santo et al., 1988) with such high variability observed by Berthou et al., (2003) in pepper. Also some European populations of *M. chitwoodi* have been shown to possess variability in virulence capable of suppressing plant defense mechanisms (Van der Beek et al., 1998). Therefore, it is important to further screen the potential of these cultivars for resistance using populations of *M. chitwoodi* from different regions. This is because the real potential of these cultivars as being resistant could be confirmed by using populations of *M. chitwoodi* in carrot producing fields having different virulence.

The occurrence of delayed development in cultivars *Berlanda* and *Parmex* could be imploied in combination with shortening of the growing season to avoid population build-up and damage of *M. chitwoodi*. This could be useful to breeders to produce better adapted cultivars by incorporating the possible resistant genes of these commercial cultivars to other commercial carrot cultivars.
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