



Wild strawberry *Fragaria vesca* as potential source for phytonematode resistance

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Accepted: 18 November 2023 / Published online: 8 December 2023
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Abstract Phytonematode infections are a major constraint to agriculture and can cause pre-harvest losses of up to one-third of the crop in affected fields. With increasing restrictions on the use of chemical pesticides due to environmental and human hazards, and increasing nematode pressure due to climate change, soil degradation and agricultural intensification, more sustainable ways to manage such plant pests are needed to meet the growing demand for food. Therefore, the reproduction of thermophilic *Meloidogyne* spp. was evaluated in comparison with that of the northern root-knot nematode *Meloidogyne hapla* on the wild strawberry *Fragaria vesca* vs. *semperflorens* cv. Alexandria, a potential source of resistance to nematode infection. *M. hapla* showed a high reproductive rate in *F. vesca*, while the thermophilic *Meloidogyne* species tested showed significant lower reproductive rates. This suggests that *F. vesca* vs. *semperflorens* cv. Alexandria, although not resistant to the nematode species tested, could be used in a management system to down-regulate nematode pressure. In addition, this study helps to reinforce the importance of crop wild

relatives in the search for resistance traits to support a more sustainable agriculture.

Keywords *Meloidogyne hapla* · *Meloidogyne incognita* · *Meloidogyne arenaria* · *Meloidogyne javanica* · *Meloidogyne enterolobii*

Short communication

Nematode problems in strawberry (*Fragaria* × *ananas*) have been reported worldwide, and as strawberry yields decline over time, nematode infestations can significantly accelerate this process (Abd-Elgawad, 2019; LaMondia, 1999). Several species of plant parasitic nematodes such as lesion nematodes (*Pratylenchus penetrans*), needle nematodes (*Longidorus* sp.), dagger nematodes (*Xiphinema* spp.), foliar and stem nematodes (*Aphelenchoides* sp. and *Ditylenchus dipsaci*), and root-knot nematodes (*Meloidogyne* spp.), can damage strawberry plants (Brown et al., 1993; Bélair & Khanizadeh, 1994; Samaliev & Mohamedova 2011; Nyoike et al., 2012; Talavera et al., 2019).

For root-knot nematodes (RKN), *Meloidogyne hapla* is commonly reported on strawberry plants (Nyoike et al., 2012; Talavera et al., 2019). The life cycle of *M. hapla* can be completed in 5 to 6 weeks under ideal climatic conditions (Perry & Moens, 2013; Vestergård, 2019). Each female can lay hundreds of eggs in a gelatinous substance, mostly outside of the root. In the second larval stage, the

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nematodes penetrate the host plant. The larvae migrate intercellularly to the central cylinder using the mouth spear (stylet) common to plant-parasitic nematodes. There they induce the formation of a nutritive tissue by inducing multinucleate giant cells, supplying the nematodes with food for their life cycle. The now sedentary larvae molt three times before becoming adults. The female lays her eggs in a gelatinous substance, mostly outside of the root. Due to the development of the giant cells, root galls of different sizes can be seen on the roots, varying according to the host plant. Depending on climatic conditions, two to three generations may develop per year in temperate latitudes.

Little is known about tropical root knot nematodes infecting strawberry. For example, in Spain, 71% of strawberry RKN infections are caused by *M. hapla*, and other *Meloidogyne* spp. found in strawberry production fields include *M. incognita*, *M. javanica*, and *M. arenaria* (Park et al., 2005; Talavera et al., 2019;). Other locations where thermophilic *Meloidogyne* spp. have been detected on strawberries include Brazil, with *M. incognita* and *M. javanica* (Krezanoski et al., 2020), Korea, with *M. arenaria* and *M. incognita* (Park et al., 2005), and the USA (Nyoike et al., 2012), Egypt (Abd-Elgawad, 2019) and Taiwan (Chen & Tsay, 2006), with *M. incognita*.

Fragaria vesca, a diploid herbaceous perennial commonly referred to as the “wild strawberry”, or “European strawberry”, has been reported to be “resistant” to *M. incognita*, which is unable to produce eggs and juveniles on these plants (Duggal et al., 2018). *Fragaria vesca*, a member of the rose family, grows wild in much of the northern hemisphere and is of great interest for introgression of aromas into the main cultivated octoploid strawberry, *F. ananassa*, because it contains methyl anthranilate, a potent aromatic compound with highly distinctive sensory effects (Porter et al., 2023). In addition, *F. vesca* is often used as a genetic model plant for the Rosaceae family (Edger et al., 2018) and its genome was first sequenced in 2010 (Shulaev et al., 2011), and later in 2017, for an improved and more detailed sequencing (Edger et al., 2018).

Because *F. vesca* is used in basic and applied research for disease resistance (Chen et al., 2016; Wei et al., 2016), and *M. incognita* may not be able to propagate on these plants (Duggal et al., 2018), this study evaluated the propagation of thermophilic

Meloidogyne species compared to *M. hapla* in the search for a potential source of resistance to RKN.

Therefore, in this study we evaluated the reproduction of the thermophilic RKN *M. incognita*, *M. arenaria*, *M. javanica* and *M. enterolobii* and the northern RKN *M. hapla*. Nematodes (1000 J2) were inoculated in each pot (Ø 14 cm; n=7) containing one *F. vesca* plant grown in steamed soil: silver sand mix (1:3; v:v), under a day/night rhythm of 16 h/08 h, at 20 °C during the night and 22 °C during the day, in a growth chamber with 60% humidity. Six weeks after nematode inoculation, roots were washed free of substrate and placed intact (roots with crown) in a mist chamber (set at 24 °C, in the dark) for J2 extraction over 17 days. The same conditions were also used to test the effect of an inoculum of 10,000 J2 (second trial) and for comparison with a known host plant, using 4-week-old tomato plants cultivar Money Maker (third trial). Only *M. hapla* (population 1) showed a reproductive increase on *Fragaria vesca* vs. *semperflorens* cv. Alexandria that was greater than threefold, resulting in 3105.7 ± 1081.2 J2/root system (Fig. 1 A). While the other RKN, namely *M. incognita* population 1 (127.1 ± 168.3 J2/root system) and population 2 (234.3 ± 251.2 J2/root system), *M. arenaria* (232.9 ± 178 J2/root system), *M. enterolobii* (290.0 ± 338.2 J2/root system) and *M. javanica* (475.0 ± 98.4 J2/root system) had a lower reproductive rate remaining below 50% of the initial inoculation rate (Fig. 1A).

In the second trial (using 10,000 J2/pot), an additional *M. hapla* population (population 2) from a Swiss greenhouse growing mainly lettuce, cucumbers, tomatoes and peppers, which we could ensure had no previous contact with strawberry plants, was added to the experimental setup (Fig. 1B). Interestingly, both *M. hapla* populations 1 and 2 propagated significantly better than the thermophilic RKN tested. *M. hapla* population 1 had a reproduction rate of 3.33 and *M. hapla* population 2 had reproduction rate of 3.6. All thermophilic *Meloidogyne* species had a reproductive factor lower than one, with *M. incognita* population 1 having the lower reproductive rate of 0.08.

To ensure, that the inoculation rate during the experiment was consistent, and that the “low” temperature did not have an impact on the thermophilic RKN performance we applied the same number of J2 (1000) to tomato plants cv. Money Maker during the experiment for each *Meloidogyne* species and population (Fig. 1C).

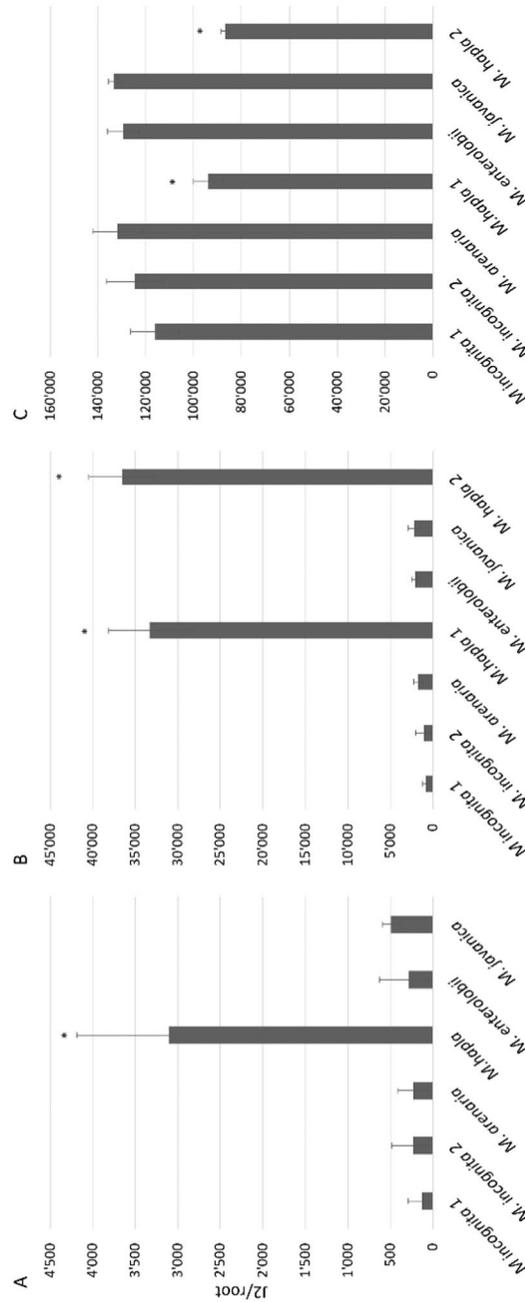


Fig. 1 Host status of *Fragaria vesca* vs. *semperflorens* cv. Alexandria for *Meloidogyne incognita* (population 1 and 2), *Meloidogyne arenaria*, *Meloidogyne javanica*, *Meloidogyne enterolobii* and *Meloidogyne hapla* (population 1 and 2), by evaluating the hatching rate of second stage juveniles (J2) from the root systems. six weeks after inoculating each pot with 1000 J2 (A) or 10,000 J2 (B) or 10,000 J2/pot (C). Host status of tomato cultivar Money Maker's for the selected *Meloidogyne* spp. was assessed to compare the hatching rate of second stage juveniles (J2) after inoculation of 10,000 J2/pot (n=7)

Reproduction results showed that all *Meloidogyne* species propagated well on the roots of tomato plants. Interestingly, both *M. hapla* populations had the lower reproductive rate on tomato compared to the other *Meloidogyne* species. However, with over 80,000 J2/ roots harvested, the reproductive rate of *M. hapla* was more than eightfold.

Despite the fact that all RKN were able to reproduce on the strawberry *Fragaria vesca* vs. *semperflorens* cv. Alexandria, the results are important to study the differences of the northern RKN *M. hapla* compared to the tested thermophilic RKN *M. incognita*, *M. arenaria*, *M. javanica* and *M. enterolobii*. The reasons for the decline in reproduction of thermophilic RKN are unclear.

Based on the RKN base temperature (Tb) of approximately 10 °C for most tropical RKN (*M. arenaria* = 10.3 °C, *M. incognita* = 9.8 °C, and *M. javanica* = 10.6 °C) (Dávila-Negrón & Dickson, 2013), and 8.25 °C for *M. hapla*, the average accumulated degree days (DD) were 487.2 °C for the thermophilic RKN and 560.7 °C for *M. hapla* during the growth period in the growth chamber. An additional 267.75 °C DD and 238 °C DD, respectively, for the development of the thermophilic *Meloidogyne* species and *M. hapla* eggs in the mist chamber resulted in a total DD of 725.5 °C DD for thermophilic RKN and 828.45 °C DD for *M. hapla*.

Since the natural base temperature is usually higher for thermophilic RKN, this may have affected the plant-nematode interaction during the experiment. Interestingly, Trudgill and Perry estimated a switching point of 21 °C, below which the temperate *M. hapla* with a shorter life cycle had a species advantage over *M. javanica*, and above which the tropical RKN had its competitive advantage. The average day and night temperature in the growth chamber was 21.6 °C.

As reported in the literature for *M. enterolobii* and *M. incognita*, 308.3 °C DD and 248.1 °C DD, respectively, are required for the development of an egg-laying female, and after 506 °C DD (*M. enterolobii*) and 552.3 °C DD (*M. incognita*) new-vermiform J2 were observed (Velloso et al., 2022). While for *M. hapa* (8.25 °C Tb) it is estimated, that 554 °C DD are required (Trudgill & Perry, 1994). Therefore, the DD achieved during the experimental setup was sufficient for the development of all RKN tested. In addition, based on the RKN propagation on the tomato plants,

we were confident that there was no limiting effect on the temperature required for the thermophilic RKN to successfully develop and propagate. Hence, we believe that plant genetics are the cause of the impaired RKN development. It is important to note, that other studies proposed different Tb, as reported by Ploeg and Maris (1999), 10.1 °C for *M. incognita*, 13.1 °C for *M. javanica*, and Ferris et al. (1978), 10.11 °C for *M. arenaria*, which would consequently result in different DD as presented here. However, it can be suggested that these differences reported in Tb may be due to differences in the populations of *Meloidogyne* spp. tested or it may be due to discrepancies in thermometer calibration.

With the sequencing and new mapping of the *Fragaria vesca* genome opening up new opportunities for further molecular investigation, a source of RKN resistance may be found in *Fragaria vesca*. This is especially true as previous investigations have shown no reproduction of *M. incognita* on *Fragaria vesca* (Duggal et al., 2018). Furthermore, seven different strawberry (*F. ananassa*) genotypes that have been tested against the emerging RKN *M. enterolobii* were non-hosts (Freitas et al., 2017). To the best of our knowledge, no previous research has used the *F. vesca* cultivar Alexandria. Therefore, we can only hypothesize that the resistance mechanisms may have been altered compared to its wild relative, such that we had RKN progeny for all *Meloidogyne* species tested. On the other hand, Cobon and Trott (2004) reported, that *M. arenaria*, *M. incognita* and *M. javanica* reproduced at high rates on the strawberry cultivar Joy, and at lower rates on other strawberry cultivars tested, Jewel and Sweet Charlie.

In Florida, where tropical RKN are prevalent, *M. hapla* is still the main *Meloidogyne* species found in strawberry production (Brito et al., 2008; Nyoike et al., 2012), and one could speculate that they may have potentially co-evolved over time. However, in the case of *M. incognita* infection, no significant damage to strawberries has been reported to date (Nyoike et al., 2012). This finding is in line with the Best4Soil Aaltjesschema, which shows the reproduction and damage on different agricultural crops (Molendijk & van Asperen, 2022). *M. hapla* has a high reproductive and damaging potential on strawberry (*Fragaria ananassa*), while *M. javanica* and *M. incognita* have a low reproductive potential, but can damage the plants.

Also, as a perennial crop, in tropical regions such as Florida, strawberries are grown as an annual crop over the winter season (Chandler et al., 2009), so *Fragaria vesca* could potentially be used to down-regulate existing thermophilic RKN before the next summer crop is planted. If grown as a perennial crop in colder climates, further studies should investigate whether *Fragaria vesca* is a non-host of *Meloidogyne fallax* or *Meloidogyne chitwoodi*, potentially controlling these quarantine nematodes over a longer period of time.

However, since strawberries are planted as transplants, it is important to ensure, that the transplants are free of *M. hapla* and other diseases. In addition to the spread of *M. hapla*, as mentioned above for *F. ananassa*, *Fragaria vesca* has also the potential to be the source of the spread of *M. incognita*, *M. arenaria*, *M. javanica* and *M. enterolobii*. It is also important to bear in mind that thermophilic RKN can still affect the crop if the initial population is high. Furthermore, it cannot be excluded that the thermophilic RKN nematodes tested, *M. incognita*, *M. arenaria*, *M. javanica* and *M. enterolobii*, may overcome the adversity and be able to develop on strawberries in the future as well as *M. hapla*.

In conclusion, this study adds to an increasing number of reports showing that crop wild relatives in general, such as wild strawberry as presented here or wild potato for example, contain genetic resources that could contribute to breeding for phytonematode resistance in modern crops and to a more sustainable crop production.

Funding Open access funding provided by Agroscope

Declarations

Conflict of interest The authors declare no conflict of interest.

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