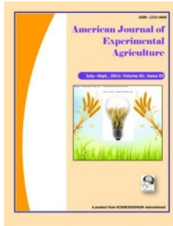

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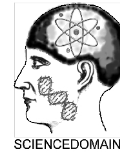

Host Suitability of Cut-Flowers to *Meloidogyne spp.* and Population Dynamics of *M. hapla* on the rootstock *Rosa corymbifera* ˈLaxaˈ

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Authors' contributions

This work was carried out in collaboration between all authors. Author BHM designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors HWD and JH helped with the experimental layout contributed to the literature searches and managed the final editing. All authors read and approved the final manuscript.

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ABSTRACT

The host suitability of cut-flowers to *Meloidogyne spp*. was tested under greenhouse conditions. In an experiment, the reaction of seven cut-flower species *viz. Dianthus plumarius, Dianthus caryophyllus, Gypsophila paniculata, Limonium sinuatum* (Fortress Dunkelblau)*, Limonium sinuatum* (Petite Bouquet Mix), *Rosa corymbifera* ˈLaxaˈ and *Freesia laxa* against the root-knot nematode *Meloidogyne hapla* and *M. incognita* was evaluated. There were significant (*P*<0.001) differences in plant species as host for either *M. hapla* or *M. incognita*. *Freesia laxa* appeared to be a poor host for *M. hapla* and *M. incognita* with a reproductive factor of 0.5 and 1.1, respectively. *Gypsophila paniculata* and *Rosa corymbifera* were not suitable hosts for *M. incognita* resulting in a reproductive factor below one. On the other hand *M. hapla* reproduced significantly (*P*<0.05) higher on *R. corymbifera* ˈLaxaˈ than on the other plant species assessed. In all plant species, nematode infected plants were less vigorous than their uninfected controls. In the second test, the pathogenicity and population dynamics of *M. hapla* on the rootstock *R.*

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corymbifera ˈLaxaˈ were demonstrated. Within 24 hours after inoculation, about 2% of the juveniles had penetrated the root system. A week later, nematode penetration reached 14%. First eggs appeared 43 days after root infection. At final termination of the experiment 78 days after inoculation the reproduction factor of *M. hapla* was 58.9. In infected plants number of leaves per plant was lower than in the respective controls. In conclusion, the tested flower plants were hosts for *M. hapla* and *M. incognita*; however, the host status varied between plant and nematode species. *R. corymbifera* ˈLaxaˈ turned out to be a very good host for *M. hapla* allowing high nematode reproduction.

Keywords: Roses; root-knot nematodes; reproduction factor; plant growth.

1. INTRODUCTION

Within the past decade, Ethiopia developed to one of the main cut-flower producing countries in East Africa. About 80% of all cut flowers produced are roses, mainly for export to Europe. Other cut flower species include carnation, statice, gypsophila and freesia. While fungal diseases and insects are already considered as a major pest problem on cut flowers in Ethiopia [1], plant-parasitic nematodes have been ignored for a long time. Only recently, thirteen genera of plant-parasitic nematodes were reported to be associated with cut flowers produced in Ethiopia [2].

Among plant-parasitic nematodes, root-knot nematodes *(Meloidogyne* spp.) are generally considered the economically most important group worldwide [3]. Three of the most common tropical species, i.e. *M. incognita*, *M. ethopica* and *M. javanica*, are known to occur in Ethiopia [4], although they have not yet been detected on cut flowers. Recently, *Meloidogyne hapla* was found to be the most frequent and abundant species in rose greenhouses in Ethiopia [5]. For temperate regions, the damage potential of *M. hapla* on cut flowers is well documented throughout the world [6-10]. However, little is known about the damage potential of *M. hapla* on cut flowers other than roses as well as of the tropical root-knot nematode species on cut flowers grown in Ethiopia. A good understanding of the host suitability of cut-flower species grown in Ethiopia to *M. hapla* and *M. incognita* is essential for future management strategies.

Therefore, the objective of the present study was to i) Evaluate the host status of seven cut-flower species/cultivars to *M. hapla* and *M. incognita* and to ii) Describe the pathogenicity and development of *M. hapla* on the most commonly grown rootstock *R. corymbifera* 'Laxa' over time.

2. MATERIALS AND METHODS

2.1 Plant Material and Growth Condition

In experiment 1 the following cut-flower species were evaluated: Carnation (*Dianthus plumarius* and *Dianthus caryophyllus*), gypsophila (*Gypsophila paniculata*), statice (*Limonium sinuatum* cv. Fortress Dunkelblau and *L. sinuatum* cv. Petite Bouquet Mix), rose (*Rosa corymbifera* ˈLaxaˈ) and freesia (*Freesia laxa*). *Rose corymbifera* ˈLaxaˈ seeds were provided by Klei (Heidgraben, Germany), while bulbs of freesia and seeds of the other species were obtained from Volmary GmbH (Münster, Germany).

Seeds were germinated in plastic trays filled with growth substrate (Floragard®, Oldenburg, Germany). Two weeks after germination, individual seedlings were transplanted into 75ml multi-well plates filled with steam-sterilized field soil. One month old seedlings were finally transplanted into 1 l capacity plastic pots filled with steam-sterilized field soil and silver sand (2:1, v:v), respectively. Bulbs of freesia were directly planted into the plastic pots. Each cut-flower species/cultivar was inoculated with either *M. hapla*, *M. incognita* or left uninoculated (control). Each treatment was replicated 10 times and pots were randomly arranged in the greenhouse at about 20±3ºC. The photocycle was adjusted to 16 h light and 8 h dark period using 600 W, 58500 lumen lamps (Norka-Lighting®, Hamburg, Germany). Plants were watered daily as needed and once weekly fertilized with 0.3% WUXAL® Super liquid foliar fertilizer (Agrarversand Oberland, Schongau, Germany).

In the second experiment the pathogenicity and population dynamics of *M. hapla* on *R. corymbifera* 'Laxa' was assessed. Non-inoculated plants served as control. Planting procedure and growth conditions were the same as described above. Sampling dates were 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71 and 78 days after nematode inoculation (DAI). Each treatment was replicated 5 times.

2.2 Nematode Inoculation

A stock population of both *M. hapla* and *M. incognita* originating from Germany was maintained on tomato (*Solanum lycopersicum* cv. Moneymaker) in the greenhouse. For collecting juvenile inoculum, galled tomato roots were placed in a misting chamber as described by [11]. Freshly hatched second-stage juveniles (J2) were collected every other day in a 1 l glass beaker and stored at 6°C. Only juveniles less than two weeks old were used in the experiments. For inoculation the nematode density was adjusted to 200 J2s per ml by adding tap water. Four vertical holes about 6 cm deep and 1 cm wide were bored in the soil around the plant stem using a rounded conical-end plastic stick. Then, 5 ml of the nematode suspension per plant was injected into the four holes using a Multipette® plus dispenser (Eppendorf®, Hamburg, Germany). Hence, each pot received a total of 1000 J2s. Control plants received a similar volume of tap water without nematodes. In Experiment 1 pots were inoculated either with *M. hapla*, *M. incognita* or remained untreated (control), in Experiment 2 pots were inoculated with *M. hapla* or remained untreated (control).

2.3 Assessments

Experiment 1 was terminated nine weeks after nematode inoculation. Shoots were cut off at soil level and fresh weight was recorded. Roots were then washed free from the adhering soil, carefully blotted on tissue paper and weighed. Eggs were dislodged from the root system using a 0.52% sodium hypochlorite (NaOCl) solution following the method described by [12] J2 numbers in the soil were recovered from 100 ml soil aliquot using the modified Baermann technique [11]. Both eggs and J2 were counted at 63× magnification using a compound microscope. The final population was determined as the sum of eggs extracted from the roots and J2s extracted from the soil.

Within the second experiment the first sampling was taken one day after inoculation. The remaining eleven samples were taken every seven days until 78 DAI. At each sampling date, shoot height, leaf number, shoot fresh weight, root fresh weight and number of galls was recorded. For the first six sampling dates until 36 DAI, nematodes within the root system were directly counted under a stereomicroscope after staining with 2% acid fuchsin [13]. For

the final six sampling dates from 43 DAI to 78 DAI, number of eggs and J2s extracted from the roots using a 2% hypochlorite solution and number of J2s from soil extracted by Baermann were evaluated. In addition, symptoms such as wilting and leaf chlorosis were visually assessed.

2.4 Statistical Analysis

In the first experiment, shoot fresh weight and root fresh weight, final nematode population and nematode multiplication factor (RF) were subjected to analysis of variance followed by mean separation ($P \leq 0.05$) with Duncan's multiple-range test using STATISTICA 7 software [14]. Each plant species or cultivar was ranked for 'host status' on the basis of the nematodes reproductive factor (RF=Pf/Pi) according to [15]: Poor host ($0<RF<1$), maintenance host ($RF \approx 1$), good host ($1<RF<10$) and excellent host ($RF \geq 10$).

In the second experiment, all plant growth parameters, final nematode population densities (Pf), number of galls per root system and reproduction factors were subjected to analysis of variance taking number of days after inoculation as a factor. Means were compared as described above. Linear models were estimated to the relationship between gall numbers per root system and weeks after nematode inoculation as well as for nematodes in the root and days after nematode inoculation.

3. RESULTS

3.1 Experiment 1: Host Plant Suitability

Cut-flower species and cultivars significantly differed ($P<0.001$) in their host suitability to either *M. hapla* or *M. incognita* (Table 1). For *M. hapla*, *R. corymbifera* 'Laxa' was found to be the most susceptible host with RF=12.7, followed by *L. sinuatum* cv. Fortress Dunkelblau with RF=9.2. On the contrary, *F. laxa* was rated nonhost or poor host with RF=0.5. The other cut flower species and cultivars were good hosts for *M. hapla* with RF's varying between 2.0 (*G. paniculata*) and 7.1 (*L. sinuatum* cv. Petite Bouquet Mix).

Regarding *M. incognita*, the most susceptible cut-flower species was *D. plumarius* with RF = 7.2 (Table 1), followed by *D. caryophyllus* (RF=6.7) and *L. sinuatum* (RF=6.2). In contrary, *R. corymbifera* 'Laxa' (RF=0.6) and *G. paniculata* (RF=0.8) were non hosts to poor hosts for *M. incognita. Freesia laxa* (RF=1.1) turned out to be maintenance host.

Infestation with *M. hapla* significantly ($P<0.05$) reduced shoot and root fresh weight of *R. corymbifera*, *D. plumarius* and *D. caryophyllus* and root fresh weight of *F. laxa* in comparison with their respective controls (Table 1). In all other cases, except for shoot fresh weight of *L. sinuatum* cv. Petite Bouquet Mix, shoot and root fresh weight in nematode infested plants was less than in non-infested plants, although not significantly. For *M. incognita* the nematode effect on plant growth was less pronounced (Table 1). A significant reduction was only observed for shoot and root fresh weight of *D. plumarius* and shoot fresh weight of *F. laxa*. Although shoot and root fresh weight of all other flower crops (except for shoot fresh weight of *L. sinuatum* cv. Petite Bouquet Mix) was less in nematode-infested plants compared to non-infested plants, albeit differences were not significant.

Table 1. Host suitability of selected cut-flower species and cultivars to *Meloidogyne hapla* and *M. incognita* under greenhouse conditions

Plant species	Inoculation	Shoot fresh weight (g)[a]	Root fresh weight (g)[a]	Nematodes per g root fresh weight	Reproductive factor (RF)[b]	Host rating[c]
Dianthus plumarius	Control	5.8±0.31[b]	8.9±1.11[b]	-	-	-
	M. incognita	4.1±0.28[a]	5.2±0.81[a]	143.3±19[cd]	7.2±0.6[c]	G
	M. hapla	4.2±0.25[a]	5.6±0.82[a]	99.3±21.2[bc]	2.5±0.8[ab]	G
Dianthus caryophyllus	Control	4.6±0.14[b]	9.5±0.90[b]	-	-	-
	M. incognita	3.9±0.09[ab]	7.6±0.96[b]	5.3±1.2[a]	6.7±0.5[c]	G
	M. hapla	3.7±0.18[a]	4.3±0.91[a]	69.3 ±17 [b]	3.9±0.9[b]	G
Gypsophila paniculata	Control	2.9±0.22[a]	9.0±0.80[a]	-	-	-
	M. incognita	2.6±0.16[a]	7.2±0.36[a]	8.0 ±2.0[a]	0.8±0.4[a]	P
	M. hapla	2.6±0.09[a]	7.4±0.96[a]	7.3 ±0.9[a]	2.0±0.5[ab]	G
Limonium sinuatum (Fortress Dunkel Blau)	Control	4.0±0.27[a]	7.0±0.38[b]	-	-	-
	M. incognita	3.6±0.19[a]	4.8±0.59[a]	133.0±17.2[c]	4.9±0.9[b]	G
	M. hapla	3.4±0.21[a]	5.3±0.67[ab]	182.7±7.5[d]	9.2±2.5[d]	G
Limonium sinuatum (Petite bouquet Mix)	Control	4.0±0.28[a]	8.1±1.38[a]	-	-	-
	M. incognita	4.1±0.21[a]	6.9±0.97[a]	75.3±9.4[b]	6.2±1.4[c]	G
	M. hapla	4.2±0.20[a]	7.6±0.98[a]	102.3 ±9.9[c]	7.1±0.9[cd]	G
Rosa corymbifera 'Laxa'	Control	11.0±1.85[b]	11.1±1.66[b]	-	-	-
	M. incognita	9.0±1.14[b]	9.2±0.98[ab]	14.3 ±2.0[a]	0.6 ±0.1[a]	P
	M. hapla	6.0 ±0.57[a]	6.7±0.43[a]	357.0± 20.1[e]	12.7±1.8[e]	E
Freesia laxa	Control	8.5 ±1.1[b]	5.3±0.6[b]	-	-	-
	M. incognita	6.4 ±1.8[a]	3.3±0.7[ab]	59.7±10.7[b]	1.1±0.6[a]	M
	M. hapla	8.1 ±1.5[b]	2.7±0.6[a]	46.0±15.0[ab]	0.5±0.2[a]	P

Data are means of ten replicates. Means in columns followed by the same letter do not differ ($P \le 0.05$) according to Duncan's multiple-range test.
[a]Means were compared with the uninfected control and infected plants of the same species or cultivars.
[b]RF = Reproduction factor, i.e. final nematode density/initial nematode density
[c]Host status category: poor to nonhost (P) ($0<R<1$); maintenance (M) (≈ 1); good (G) ($1<R<10$); and excellent (E) ($R>10$)

Table 2. Plant growth performances and nematode numbers over time on *Rosa corymbifera* 'Laxa' infested by *Meloidoyne hapla*

DAI	Treatment	Leaf Nr.*	Shoot fresh weight (g)	Root fresh weight (g)	Shoot height (cm)	Total number of nematode per root system	Final nematode population (soil and root)	RF (Pf/Pi)
01	Control	4.6±0.40^{l}	0.2±0.02 ^{k}	0.2±0.02^{k}	5.10±0.29^{j}	-	-	-
	infected	5.8±0.37^{k}	0.4±0.03 ^{k}	0.2±0.03^{k}	5.3±0.26^{j}	13.8±2.9^{d}	-	-
08	Control	7.0±0.32^{j}	0.6±0.04 ^{j}	0.3±0.03^{k}	7.1±0.34^{i}	-	-	-
	infected	8.2±0.58^{i}	0.6±0.09 ^{j}	0.3±0.05^{k}	7.2±0.45^{i}	139.2±31.9^{b}	-	-
15	Control	11.2±0.58^{h}	1.4±0.12^{i}	0.8±0.08 ^{j}	13.2±0.67^{h}	-	-	-
	infected	8.4±0.24^{i}	1.2±0.07^{i}	1.1±0.10 ^{j}	12.8±0.45^{h}	144.2±16.3^{b}	-	-
22	Control	11.8±0.80^{gh}	2.1±0.08^{h}	1.2±0.09 ^{j}	17.8±0.44^{g}	-	-	-
	infected	11.8±1.16^{gh}	1.9±0.10^{h}	1.6±0.17^{i}	16.5±0.91^{g}	124.8±17.1^{b}	-	-
29	Control	13.2±1.56^{defg}	2.9±0.15^{g}	2.7±0.18^{h}	20.7±0.83^{f}	-	-	-
	infected	12.0±0.77^{gh}	2.3±0.20^{h}	2.4±0.15^{h}	20.1±0.92^{f}	232.6±45.7^{a}	-	-
36	Control	13.8±1.16 ^{df}	3.5±0.23^{f}	3.9±0.21^{g}	22.2±0.32^{e}	-	-	-
	infected	12.2±0.37^{gh}	2.9±0.15^{g}	3.5±0.40^{g}	24.3±0.29^{d}	216.8±23.2^{a}	-	-
43	Control	14.0±0.55^{d}	3.6±0.21^{f}	6.5±0.47^{e}	25.3±0.68^{cd}	-	-	-
	infected	12.4±0.24^{gh}	3.1±0.08^{g}	5.22±0.43^{f}	25.7±0.55^{c}	-	6094±1111.3^{d}	6.1±1.1^{d}
50	Control	15.2±1.98^{bcd}	3.7±0.24^{f}	7.5 ±0.81^{de}	26.6±0.95^{c}	-	-	
	infected	13.8±0.80^{d}	4.0±0.25^{de}	6.98±0.46^{de}	28.3±1.1^{bc}	-	22610±5789.8^{cd}	22.6±5.8cd
57	Control	15.6±1.03^{c}	4.6±0.17^{b}	7.4±0.49^{d}	28.2±0.51^{b}	-	-	-
	infected	14.2±0.73^{d}	4.8±0.15^{b}	7.62±0.54^{d}	30.5±0.80^{a}	-	35546±4972.5^{bc}	35.5±4.9bc
64	Control	16.0±0.55^{c}	5.1±0.32^{ab}	8.8±0.22^{c}	29.5±0.72^{a}	-	-	-
	infected	13.2±0.49^{ef}	4.3±0.18^{cd}	8.48±1.05^{cd}	30.2±0.93^{a}	-	35896±11765.2^{bc}	35.9±11.7^{bc}
71	Control	17.4±0.87^{ab}	4.5±0.41^{bc}	9.4±0.46^{bc}	29.8±0.46^{a}	-	-	-
	infected	13.0±0.32^{f}	5.0±0.26^{ab}	10.02±0.50^{ab}	30.1±1.08^{a}	-	45725±7066.5^{ab}	45.7±7.1^{ab}
78	Control	18.4±0.49^{a}	5.6±0.40a	9.9±0.42^{b}	31.1±0.88^{a}	-	-	-
	infected	12.2±0.37^{g}	4.2±0.19^{d}	10.8±0.24^{a}	30.8±1.73^{a}	-	58930±5460.8^{a}	58.9±5.5^{a}

** All measurements are means ± standard deviation. Means in columns followed by the same letter do not differ (P≤0.05) according to Duncan's multiple-range test (n=5)*

3.2 Experiment 2: Population Dynamics and Pathogenicity of *Meloidogyne hapla* in *Rosa corymbifera* 'Laxa'

Within the first 36 days following inoculation, number of *M. hapla* in the root system of *R. corymbifera* 'Laxa' significantly increased ($P<0.001$) over time from 13.8 the day after inoculation to about 216.8 at 36 DAI (Table 2 above). At 36 DAI, root galls were apparent throughout the root system. At each sampling date, gall counts per root system exceeded 100 (Fig. 1). The female had reached its typical sac-like shape (data not shown) within 36 DAI. First eggs were extracted from roots at 43 DAI. Since then, number of extracted eggs increased over time until final determination at 78 DAI when a reproductive factor of 58.9 was reached (Table 2). The nematode density extracted from roots significantly ($P<0.001$) increased over time (Fig. 2). This increase in nematode density over time was found to have a moderately positive relationship with root fresh weight ($r^2=0.342$; $p=0.001$). J2 density in the soil also significantly ($P=0.003$) increased over time (Fig. 3). However, no statistically significant relationship was found between nematode eggs extracted from the root and juvenile population density in the soil.

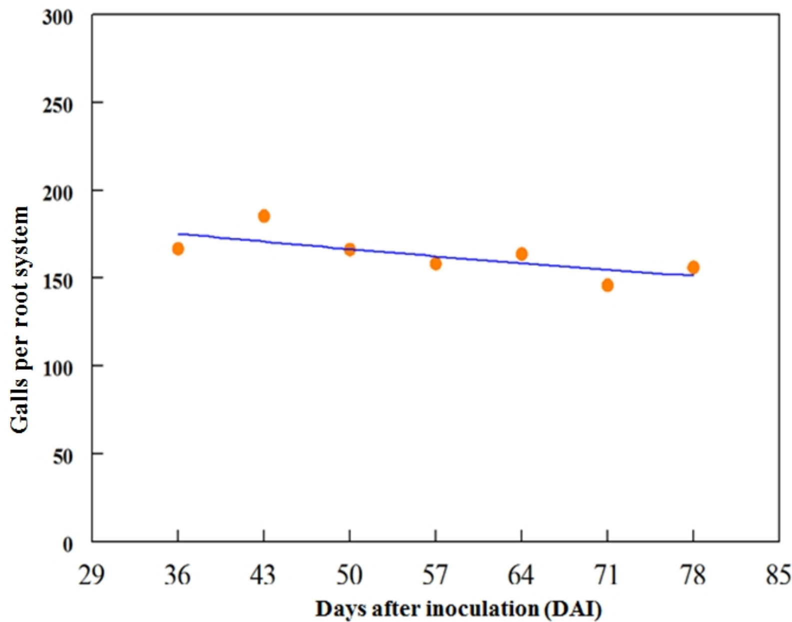

Fig. 1. Linear relationship between root gall number and time after nematode inoculation (n=5)

Infected plants showed reduced growth compared to uninfected control, especially towards the end of the experiment (Table 2). For instance, the number of leaves per plant was significantly ($P<0.05$) less over time. Moreover, leaves of infected plants had a typical shrivelled margin, yellow blade and petiole and shortly dropped-off. No significant difference in shoot fresh weight was observed between 50 and 71 days after nematode inoculation. However, a significant reduction ($P<0.001$) in shoot fresh weight was recorded in infected plants compared with uninfected plants at the final sampling date (Table 2). Uninfected plants showed a consistent increase in shoot height over time until the final date of harvest. On the other hand, infected plants revealed a significant ($P = 0.03$) increase in plant height

only until 50 days, after which no significant increase was found. At the end of the experiment, the difference in plant height between infected and uninfected control plant is shown (Fig. 4A and B). No significant difference in root fresh weight between infected and uninfected plants was found until 57 DAI. However, at 64 DAI, roots were heavier (*P*<0.001) than those of uninfected plants. Visually, roots of infected plants were shorter and bushier (Fig. 4C and D). In addition, infected roots were greyish and had a very loosen root bark.

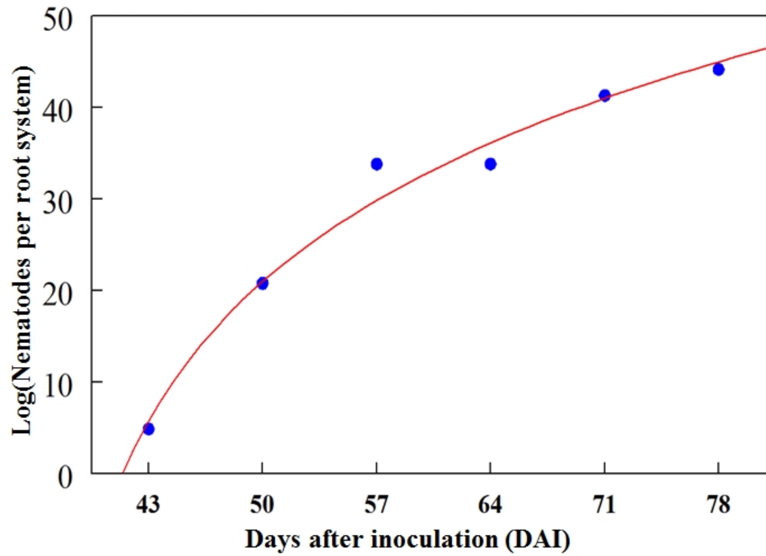

Fig. 2. The relationship between nematodes per root system and days after inoculation (n=5)

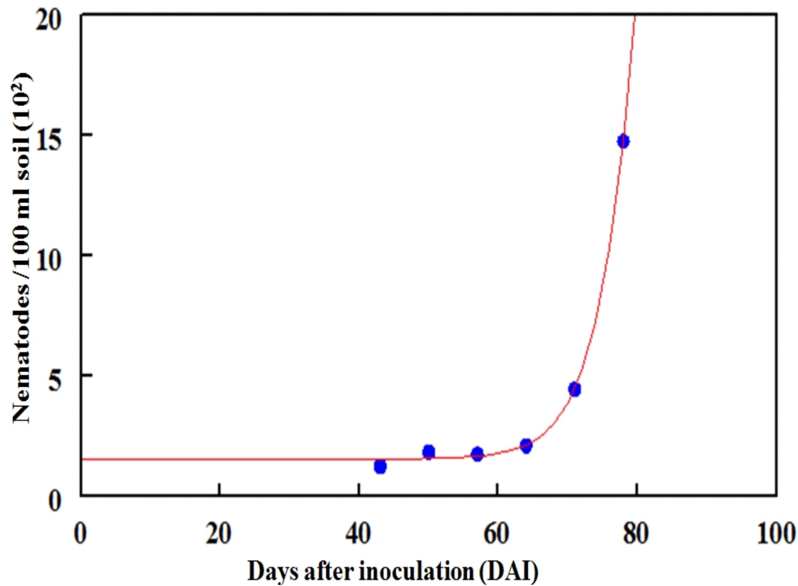

Fig. 3. Population dynamics of *Meloidogyne hapla* in the soil 43-78 days after plants were inoculated (n=5)

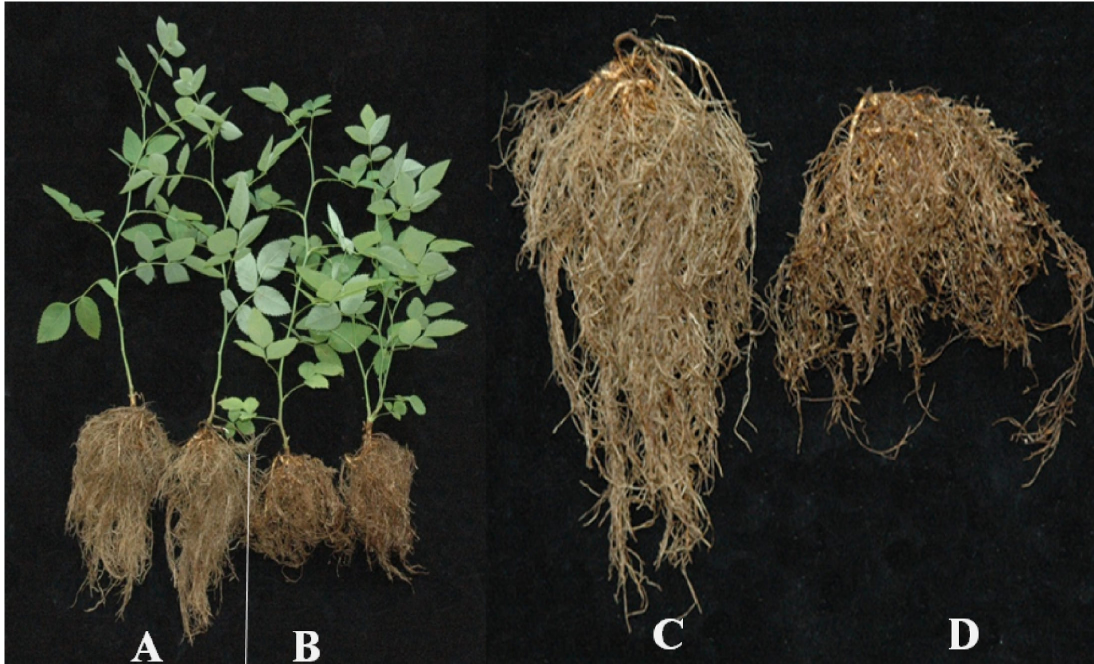

Fig. 4. Effect of *Meloidogyne hapla* infection on growth of *Rosa corymbifera* ˈLaxaˈ 78 days after nematode inoculation. Uninfected control (A and C) and infected plants (B and D)

4. DISCUSSION

Over the last decade, Ethiopia developed to a major producer of cut flowers for the international market. Meressa et al. 2012 detected plant-parasitic nematodes associated with roses grown in greenhouses in Ethiopia. Infested greenhouse sites were often associated with poor plant stand. The question rose, if common root-knot nematodes species such as *M. hapla* and *M. incognita* could also multiply on cut flower species grown in Ethiopia. Both scenarios would mean a major threat to cut flower production in Ethiopia.

All cut flower species and cultivars tested in this study turned out to be good or excellent hosts for *M. hapla* except for *F. laxa*, and good hosts for *M. incognita*, except for *R. corymbifera* ˈLaxaˈ and *G. paniculata*. However, there was a substantial difference in host status of the tested cut-flower species and cultivars for both root-knot nematode species. Unfortunately, none of the tested species and cultivars was resistant for both nematode species at the same time. In general, rotation between host and nonhost crops is an important component of IPM of plant-parasitic nematodes [16] and knowing the host status of any given crop might enable the farmer to use this crop for nematode management. However, this tool might be of limited value if cut-flowers are produced in monoculture.

In our study *R. corymbifera* ˈLaxaˈ was a host for *M. hapla*. This is confirmed by [6] who tested 13 rose rootstocks in the field and all rootstocks turned out to be hosts for *M. hapla*, except for *Rosa canina* cv Success and *R. canina* cv Heinsohn's Rekord, being poor hosts. Similarly, [10] found all nine rootstocks of *Rosa multiflora* and *R. indica* being hosts for *M. hapla*, although at variable degree. However, it should not be ignored, that the host plant

response can be influenced by the origin of the *M. hapla* isolate. This was shown by [17] for rose rootstocks of *R. multiflora* and *R. indica* using 4 geographic isolates of *M. hapla*. In their studies host suitability of *R. multiflora* clone K1 ranged from intermediate to resistant depending on the *M. hapla* isolate used. Similarly, *R. indica* was a good to excellent host for a specific isolate of *M. hapla* from Canada but resistant to three other isolates, thus expressing an isolate-specific resistance. This clearly shows the importance of testing host plant suitability under local conditions. Quite interestingly, *R. corymbifera* 'Laxa' was a poor to nonhost for *M. incognita*. If this can be confirmed under field conditions, *R. corymbifera* 'Laxa' could be a useful tool to control *M. incognita* at infested sites.

In our study, both species of carnation and both cultivars of *Limonium* were good hosts for *M. hapla* and *M. incognita.* However, host suitability varied between plant genotype and nematode species, e.g. both carnation species allowed a higher reproduction of *M. incognita* than of *M. hapla*. For carnation, *M. incognita* is considered the main cause for yield reduction estimated to reach 20% worldwide [18]. Differences in host status among carnation cultivars for *M. incognita* have been reported by [19]. The 33 screened cultivars fall into three groups ranging from highly and moderate resistant to susceptible.

Freesia laxa was a poor host for *M. hapla* and a maintenance host for *M. incognita*. For both nematode species, only few small galls were observed on freesia roots. However, numerous small dark necroses were associated with the bulbs. Unfortunately, no information on *M. hapla* and *M. incognita* affecting freesia was found in the literature. However, for another tropical root-knot nematode species, *M. javanica*, [20] reported that infected roots showed light galling and plants suffered a lot even at low densities of *M. javanica*. If the poor host status of freesia can be used in the field to reduce high densities of *M. hapla* or *M. incognita* still needs to be proofed. In commercial rose production, there is usually a short fallow period between two consecutive crops in which old plants are uprooted and left to dry on the soil surface. This fallow period might be used to grow resistant short season freesia before new rose seedlings are transplanted.

As shown here *G. paniculata* was a poor host for *M. incognita* and only allowed little multiplication (RF=2.0) of *M. hapla*. In contrast, [21,22,23] found *G. paniculata* being susceptible to *M. incognita*. This discrepancy might be attributed to differences in the used nematode isolate or cultivar as discussed above.

Root invasion, development and duration of the life cycle of *M. hapla* depend on both host plant species and environmental conditions [24]. Within this respect, the development and population dynamics of *M. hapla* was studied more in detail on *R. corymbifera* 'Laxa'. First juveniles penetrated the root tips within 24 hours after inoculation. Under the given temperature of 20±3ºC, *M. hapla* completed its life cycle between 36 and 43 DAI. Unfortunately, the exact date could not be determined as sampling was done on a weekly basis. At final termination of the experiment 78 DAI a RF=58.9 was achieved indicating the enormous reproduction potential of *M. hapla* on *R. corymbifera* 'Laxa'. Nematode infection was associated with a lower number of leaves per plant compared with uninfected controls. However, results on shoot and root fresh weights showed no clear tendency between infected and uninfected plants. Although visual inspection indicated a reduced root system in *M. hapla* infested plants, root fresh weight was for most sampling dates not significantly reduced. Most likely reduced root length was compensated by the weight of the root galls [25]. Moreover, gall numbers per root virtually decreased over time which most likely can be contributed to the fusion of neighbouring galls in older roots thereby impaired the counting.

Growing roses over several years in combination with the high reproduction rate of *M. hapla* under greenhouse conditions will facilitate *M. hapla* causing severe losses [9,26]. Although little is known about the overall economic damage [27] reported a reduction of 19,000 harvestable flower stems per ha and year. In infested soil, roots are damaged and water and nutrient uptake is disturbed resulting in wilting, leaf discoloration and senescence [28]. Our observation of leave chlorosis on nematode infested plants was most likely attributed to nutrient deficiency. As reported by [29] root-knot nematode infection can cause reduction of leaf nitrogen, an important component of leaf chlorophyll.

5. CONCLUSION

The tested cut-flower species were in general good hosts for *M. hapla* or *M. incognita*. Especially the rose rootstock *R. corymbifera* 'Laxa' turned out to be an excellent host for *M. hapla* and thus should be excluded from being used as a rootstock in *M. hapla* infested greenhouses. Therefore, *M. hapla* and *M. incognita* present a severe threat to cut-flower production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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