

A Search for the Sources of Root Knot Nematodes in Commercial Rose Nurseries

N. García Victoria and J.J. Amsing
Applied Plant Research (PPO)
Business Unit Glasshouse Horticulture
Aalsmeer
The Netherlands

Keywords: root knot nematodes, *Meloidogyne hapla*, sources, disinfection of recirculating drainage water, rose

Abstract

Between 2000 and 2002, increasing numbers of Dutch commercial hydroponic rose cultures became infested with plant parasitic nematodes, mostly by the northern root knot nematode *Meloidogyne hapla*. Affected growers reported production losses up to 40%. Chemical treatments against nematodes are not allowed for roses grown on hydroponics in The Netherlands. Therefore, prevention is the only way to protect crops from becoming infected. In order to decrease the number of rose crops infested with root nematodes, more knowledge about the sources and spread of these plant parasitic nematodes was necessary. A survey in and around eleven rose nurseries was conducted. Nematode sources and cultivation handlings that spread nematodes were identified. Hygiene protocols for tool disinfection and nematode free crop replacement were developed, implemented and tested at commercial nurseries. Two years after crop replacement following this protocol, formerly infected greenhouses were still nematode free. This survey has showed that it is viable to cultivate roses on recirculating hydroponic systems without plant parasitic nematodes. Conditions to be fulfilled are: a nematode-free growing-system, a 100% effective disinfection device to disinfect recirculating drainage water, nematode-free planting material and hygienic measures during the cultivation period.

INTRODUCTION

The switch from soil to substrate seemed to be the end of soil borne diseases for many commercial ornamental cultures. As time passed by, not only new soil borne problems arose (Amsing and Kerssies, 1992), but many of the old ones reoccurred with renewed virulence. Root nematodes belong to this last category of soil borne plant pathogens. In recent years the number of rose cultures on hydroponics which became infested with plant parasitic nematodes continually increased. Infections were mostly caused by the northern root knot nematode *Meloidogyne hapla* and occasionally by the root lesion nematodes *Pratylenchus penetrans* and *P. vulnus*. In the beginning, the presence of the nematodes in substrate cultures could easily be explained by the use of root grafts and other plant material of which roots had originally been cultivated on soil. The introduction in the early nineties of the fungus *Phytophthora* (Amsing and Kerssies, 1991) was an example of such infections transmitted through the soil particles adhered to roots of the young plants. Alternatively, the infection risks in the actual propagation practice using mostly cuttings or top grafts are low. Nevertheless, a high incidence of infections does occur. Where do these nematodes come from, where in the nursery do they hide, how do they spread, and how can we stop them from spreading? Especially in the Dutch situation, with no chemicals allowed and no known biological products to control nematodes (Stapel et al., 2002), prevention and hygiene are the only available weapons.

In order to decrease the number of rose crops infested with root nematodes, research on sources of nematodes was conducted by Applied Plant Research, Glasshouse Horticulture financed by the Dutch Horticultural Board. The objectives of this study were

(1) to detect all sources of plant parasitic nematodes in rose greenhouses, (2) to determine by which cultivation activities these nematodes could be spread and (3) to point out which measures should be taken to prevent an attack by plant parasitic nematodes and to avoid them from spreading.

MATERIALS AND METHODS

Nursery Selection for Source Survey

Eleven commercial rose nurseries that met the four following criteria were selected to participate in the survey:

- The nursery was infected by nematodes
- The young plants were propagated through cuttings or top grafts
- The nursery re-used the drainage water (recirculating system)
- The collected drainage water was disinfected prior to re-use.

Samples were taken from different spots in the greenhouse identified as possible sources: rain water reservoir, drain water collection points, drain water silo before disinfection, drain water silo after disinfection, roots, soil (paths or uncovered areas below the raised cultivation system), debris on weed control fabric and the ditches around the greenhouse.

Propagation Facilities Survey

Although the actual propagation methods by cuttings and top grafts nearly exclude infection risks, unhygienic working methods or contact with roots (from certain rootstocks or propagation by root grafting) can lead to infection of the young plants. To determine whether young plants could be considered a nematode source for commercial nurseries, a similar survey to those at nurseries was conducted among 4 commercial propagation facilities. In total 90 samples were taken and analyzed for nematodes: substrate (coco peat), drainage water from the collection sinks and the rooting tables, water squeezed from the rock wool blocks, water from cleaned rooting tables and water with substrate and plant residues from the table cleaning machines.

Survey of Cultivation System and Sampling Activities

As well as commercial nurseries, a previously (artificially) infected rose culture in a trial greenhouse at Applied Plant Research was involved in the research in order to examine the presence of nematodes in the cultivation system (gutters and drippers).

Samples were also taken of the nutrient solution, the substrate (coco peat) and plant roots to measure the spread of nematodes by means of routine sampling tools (soil probe, suction syringe and hands).

Nursery Selection for Implementation and Evaluation of the Hygiene Protocol

Crop replacement (on average every 4 years) offers the chance to start a new cropping cycle with a nematode free cultivation system. A hygiene protocol was developed by checking, disinfecting and rechecking the presence of nematodes during crop replacement at a commercial nursery on all parts of the cultivation system.

The protocol was evaluated three times:

- a) during crop replacement at one nursery (Feb. 2003)
- b) 4 to 25 months after crop replacement at eight formerly infected nurseries that had applied the proposed hygiene protocol (Nov. 2002)
- c) 12 to 36 months after crop replacement in nine formerly infected nurseries (Oct. 2003).

The nurseries were selected based on the following criteria

- The crop to be replaced was infected by *M. hapla* or *P. penetrans*
- The new crop would be planted on the old gutters
- The new young plants would be propagated by top grafting or by cuttings
- The old cultivation system would be disinfected following the hygiene protocol
- Drain water disinfection would occur only by means of UV or a heater

Samples of the drain water were taken in the first series (2002) and of the drain water and the plant roots in the second series (2003).

Sample Processing

The samples were processed differently depending on their nature:

- The 100 L reservoir water samples were filtered through a 10 µm sieve and the filters were rinsed; the water used for rinsing was analysed for nematodes.
- The 10 L drain water samples were precipitated and concentrated to 15 ml, in which the nematodes were counted.
- From the 40 gram root samples the nematodes were extracted on nematode filters.
- Soil, debris and ditch sediment samples were analysed after processing in an Oostenbrink elutriator (van Bezooijen, 1999) followed by extraction on nematode filters.
- Gutters and drippers were sampled by collection of the water used for rinsing. The nematodes were extracted during 72 hours from the suspension on nematode filters. After precipitation and concentration, the nematodes were counted.
- Sampling tools and hands were rinsed with water. The suspension obtained by rinsing was extracted on nematode filters for nematode count.
- Drain water from propagation tables was sampled during one ebb and flood irrigation cycle (2,5 L per table). At propagation facilities without ebb and flood irrigation, the drainage was sampled by squeezing 40 rock wool blocks per table until collection of 2 l water. The nematodes were extracted on nematode filters and counted.

Laboratory Trials for Tool Disinfection

Ethyl alcohol and hot water treatments were tested for disinfection of tools (knives, sampling tools like soil probe and syringe) in order to kill adhering nematodes. In the ethyl alcohol trials two *M. hapla* stages were used: J2 (second-stage juveniles) and eggs. Seventy ml of the treatment solution (0, 40, 55 and 70% ethyl alcohol) were added to 100 ml pots containing 0.5 ml nematode suspensions. After the treatment times expired (1 to 12 minutes for J2 and 8 to 120 minutes for eggs) the content of each pot was poured on a 25 µm sieve and rinsed with water to remove residues of ethyl alcohol. The sieved J2 and eggs were brought back into suspension. The effectiveness of the treatments was evaluated by means of counting the number of living J2 (those moving spontaneously or after being touched) on the day of the treatment and one day later, just in case some of them could recover from the treatment. Effectiveness of eggs-treatments was evaluated on the day of the treatment and 4 days later by counting the numbers of J2 from hatched eggs.

In the hot water trials pieces of rose roots infected with *M. hapla* and *P. penetrans* were exposed to 60°C and 80°C for 1 to 32 minutes. Immediately after the treatment times expired rose roots were cooled down to 18°C, cut into 5 mm pieces and extracted on nematode filters for 72 hours. Numbers of J2 were counted.

RESULTS AND DISCUSSION

Sources Survey at Commercial Nurseries

Table 1 shows the sources where parasitic nematodes were found at the sampled points of the commercial nurseries. The nurseries differed greatly in number of greenhouses and organization. Empty boxes indicate therefore that no samples could be taken for that particular category at a certain nursery.

1. Roots and Drainage Water. The presence of nematodes in the drainage water corresponded well with infected roots. Therefore it is advisable to sample drain water instead of roots, since root sampling is very labour intensive and destructive. However, at low infection rates and when drain water is very abundant even drain water samples can lead to false negative results (Amsing et al., 2004). Regular sampling is advised in such cases.

2. Water Reservoirs. The presence of nematodes was confirmed in 5 of the 10 water reservoirs. How the nematodes reach the water basin is not clear, but infestations of reservoirs by weed, animals and wind (Tobar and Gallardo, 1974, 1976; Carrol and Viglerchio, 1981) is not excluded because *M. hapla* has many host plants outside the greenhouse. The sediment of 6 reservoirs was analysed, but no parasitic nematodes were found in these samples. It is known that *M. hapla* can survive several weeks in water, but they are not able to reproduce. They will only be able to feed and multiply once they have invaded the root tissue. None of the participating nurseries was disinfecting the rain water by any means; this will be an important prevention measure to take in order to avoid a crop infection. Covering the basin might help, but the preventive effect of this measure has not yet been checked.

3. Drain Water Silo. In only 6 out of the nine drain water silos sampled, was the presence of nematodes confirmed. In this kind of silo, the drain water collected from all the individual greenhouses is gathered together. When not all the greenhouses are infected, the subsequent low concentration of nematodes in the silo can reduce the chances of finding them in the sample. Also, precipitation of the nematodes together with the drain water impurities is likely to occur, as nematodes were found in all the three sediment samples analysed from silos.

4. Soil and Debris. Eight soil samples were taken from five nurseries where no plants had been cultivated in the soil for ten years or longer. Nematodes were found in only one of the samples. Because the nematodes could not have survived that long in the soil, the infection must have its origin from a leak in the raised (infected) cultivation system creating a small infection risk if drippers, drip lines or tools would fall on the infected spots and be placed back in contact with the roots or the irrigation water. The debris on the weed control fabric covering the soil was sampled in three nurseries. Although nematodes could not be found in any of the samples, there is a small infection risk if the raised system would leak infected drainage water.

5. Ditches around the Greenhouse. Samples analyzed from the sediment of eleven ditches surrounding the nurseries did not show any nematode infection. However, water from ditches remains a possible source, since many authors have found root knot and root lesion nematodes in surface water (Faulkner and Bolander, 1966, 1970a, 1970b; Tobar and Palacios, 1974).

6. Disinfected Drain Water. Two nurseries used a slow sand filtration disinfection system. In both "clean water silos", nematodes were still present, proving that this disinfection system is not advisable for avoiding nematodes. The water treated by the heater did not show nematodes in any of the samples. These results confirm the results of previous work (Amsing and Runia, 2000).

Survey at Propagation Facilities

Nematodes were not detected in any of the 90 samples taken at the propagation facilities. It was found that the professional propagators take considerable hygienic measures to avoid infections with plant parasitic nematodes. One of them was even disinfecting all the irrigation water (from the rain water reservoir) prior to use by means of a UV disinfection unit. The sporadic presence (less than 1%) of soil rooted material for root grafting propagation in some of the propagation facilities involves some risk (Amsing and Keressies, 1991). Keeping all soil rooted material strictly separated from the cuttings and top grafts will presumably reduce this risk.

Survey of Cultivation System and Sampling Activities

1. Gutters. The first brushing and rinsing of the gutters where infected plants had been cultivated contained an average of 13 J2 of *M. hapla* per meter. The suspension collected after the second rinsing did not contain any nematodes. This showed that the gutters of an infected crop, if not well brushed and cleaned, are a source of nematodes for the following crop.

2. Drippers. The water used for rinsing the drippers pulled out of rock wool, perlite or

coco peat where infected plants are growing, contained on average 9 J2 of *M. hapla* per dripper, and are therefore a source that can infect a new crop when reused without disinfection.

3. Sampling Tools. Table 2 shows the average nematode counts of: the rinsed hands after root sampling, the water obtained from rinsing a sampling syringe after extracting nutrient solution out of the substrate and in the water obtained after rinsing a soil sampling probe after sampling coco peat. All rinses contained J2 of *M. hapla*. This proves that sampling tools can spread nematodes from infected greenhouses to clean greenhouses of the same nursery, but also from one nursery to the other when external samplers (i.e. from commercial laboratories) use their own tools without disinfecting them between two nurseries.

Evaluation of the Crop Replacement Hygiene Protocol

The results of the samples taken at the nurseries where the hygiene protocol was applied during crop replacement showed that fourteen out of sixteen cleaned compartments were still free of nematodes one to two years after replacement of the nematode-infested crop.

Effectiveness of Tool Disinfection Treatments

J2 treated with ethyl alcohol (55% and 70%) were killed for 100% after a treatment time of 8 minutes and at a concentration of 40% after 12 minutes. Treatment times of 1 and 2 minutes hardly killed any J2. No recovery of J2 occurred. On the contrary, eggs did recover. Directly after treatment eggs did not hatch when they were exposed to ethyl alcohol for at least 30 minutes (Fig. 1, a). However, four days later even an exposure time of 120 minutes did not inhibit hatching eggs (Fig. 1, b). A hot water treatment of one minute at 60°C was enough to kill 100% of J2 and eggs in the *M. hapla* infested roots.

CONCLUSIONS

Sources

Plant parasitic nematodes were found at nurseries in and/or on: roots, substrate, drip-pegs, gutters, drainage water, recirculation water cleaned by slow sand filters, greenhouse soil and rainwater reservoirs. No plant parasitic nematodes were detected in inorganic and organic material collected from the weed control fabric covering the soil and in the sediment of the ditches surrounding the greenhouse. Nevertheless the soil cover and surface water could be infested with nematodes. As expected, no living nematodes were found in the drainage water disinfected with the heating installation before recirculation.

No nematodes were found in any of the samples taken at rose propagating facilities; therefore, young plants are not very likely to be a source of nematodes as long as there is no contact with root grafts and strict hygienic measures are taken to avoid infections through irrigation water.

Spread

Nematodes can be spread by means of sampling activities if nematode-infested parts of the growing-system are touched by hands or tools used for sampling roots, substrate, nutrient solution and recirculation water. Sampling roots is one of the most risky activities.

Nematode Free Crop Replacement

During crop replacement, the nematode-infested growing-system has to be clean free of nematodes. A proven effective cleaning procedure has been described in the hygiene protocol, and consists of a pre-treatment with water, followed by a treatment with 4% sodium hypochlorite (active ingredient: 14% chlorine). Fourteen out of sixteen

cleaned compartments were still free of nematodes one to two years after replacement of the nematode-infested crop.

Tool Disinfection

Infested tools and drip-pegs can be cleaned of nematodes by dipping them into water of at least 60°C for one minute. Even eggs and nematodes in roots are killed by this hot-water-treatment. Treatment with 70% ethyl alcohol for two hours is insufficient to kill all the eggs of *M. hapla*.

ACKNOWLEDGEMENTS

These projects were funded by the Dutch National Product Board for Horticulture (Productschap Tuinbouw). The cooperation of all the nurseries involved as well as the propagators is also gratefully acknowledged.

Literature Cited

- Amsing, J.J. and Kerssies, A. 1991. Zetlingen en wortelenten bronnen van ziekteverwekkers: enquête *Phytophthora* bij rozen op substraat. Vakblad voor de Bloemisterij 7: 50-51.
- Amsing, J.J. and Kerssies, A. 1992. Meer duidelijkheid over wortelrot – *Gnomonia* en *Phytophthora* in roos onderzocht. Vakblad voor de Bloemisterij 32: 26-29.
- Amsing, J.J. and Runia, W.Th. 2000. Verhitting en UV-straling fnuikend voor aaltjes: manieren van waterontsmetting tegen aaltjes onderzocht. Vakblad voor de Bloemisterij 3: 46-47.
- Amsing, J.J. and Zijlstra, C. 2003. Detectie van wortelaaltjes in water: exudaten, membraanfiltratie en zeefiltratie. PPO Rapport 583.
- Amsing, J.J. and García, N. 2003. Bronnenonderzoek wortelaaltjes bij roos – Teeltbedrijven : inventarisatie en maatregelen. PPO Rapport 586.
- Amsing, J.J. and García, N. 2003. Regenwaterbassin bron van aaltjesbesmetting. Vakblad voor de Bloemisterij 32: 42-43.
- Amsing, J.J., de Jongh, M.A. and García, N. 2004. Optimaliseren bemonsteren en extraheren van wortelaaltjes in substraatteelten roos. PPO Rapport 41103602.
- Amsing, J.J. en García, N. 2004. Opmars aaltjes met hygiëne bezworen. Vakblad voor de Bloemisterij 4: 48-49.
- Amsing, J.J., García, N. and de Jongh, M.A. 2004. Bronnenonderzoek wortelaaltjes bij roos op vermeerderingsbedrijven. PPO Rapport 41111035.
- Bezooijen van, J. 1999. Oostenbrinktrechter. In: Methoden en technieken voor Nematologie. Landbouwwuniversiteit, Vakgroep Nematologie, Wageningen, 125 p.
- Carroll, J.J. and Viglierchio, D.R. 1981. On the transport of nematodes by the wind. Journal of Nematology 13 (4): 476-483.
- Faulkner, L.R. and Bolander, W.J. 1966. Occurrence of large nematode populations in irrigation canals of South Central Washington. Nematologica 12: 591-600.
- Faulkner, L.R. and Bolander, W.J. 1970a. Acquisition and distribution of nematodes in irrigation waterways of the Columbia Basin in Eastern Washington. Journal of Nematology 2: 362-367.
- Faulkner, L.R. and Bolander, W.J. 1970b. Agriculturally-polluted irrigation water as a source of plant- parasitic nematode infestation. Journal of Nematology 2: 368-374.
- Stapel, L.H.M., Amsing, J.J., Kok, L. and Leeuwen and van, A.G.J. 2002. Bestrijding *Meloidogyne hapla* bij roos in substraat. PPO Rapport GT3.2.001, 33 p.
- Tobar, A. and Palacios, F. 1974. Nematode dispersal by irrigation water. Simposio International (XII) de Nematologia, Sociedad Europea de Nematologos, 1-7 Septiembre, 1974, Granada, Spain, 103-105.
- Tobar, A. and Gallardo, M. 1974. An experimental study on the role of wind in the dispersal of some non cyst-forming nematodes. Simposio International (XII) de Nematologia, Sociedad Europea de Nematologos, 1-7 Septiembre, 1974, Granada, Spain, 102-103.

Tobar, A. and Gallardo, M. 1976. Evaluation of the role of wind in the dispersion of desiccation-susceptible plant-parasitic nematodes. *Revista-Iberica-de-Parasitologia* 36(1-2): 89-130.

Tables

Table 1. Results of the nursery survey. + = nematodes found; - = no nematodes found. Empty boxes indicate no samples were available on this nursery for this category. The colours indicate surprising results for each sampled source.

Sources	Nursery										
	1	2	3	4	5	6	7	8	9	10	11
greenhouse 1: roots / drain water	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+			
greenhouse 2: roots / drain water	+/+	+/+	-/+	+/-	-/+		+/+	+/-			
greenhouse 3: roots / drain water		-/-	-/-		-/-		-/+	-/+			
greenhouse 4: roots / drain water								-/+			
greenhouse 5: roots / drain water								-/-			
greenhouse 6: roots / drain water								-/-			
water in drainage silo	-	+	+	+	-	-	+	+	+		
sediment in water drainage silo	+		+				+				
water after sand filter					+				+		
water after heater	-	-	-	-		-		-			
sediment in silo after heater				-							
water in rain water reservoir	-	-	+	+	-	+	-	-		+	+
sediment in rain water reservoir	-		-	-	-	-	-				
greenhouse soil	+				-	-	-	-			
debris on weed control cover		-		-							
Sediment in ditches around nursery	-	-	-	-	-	-					

Table 2. Infection level of the rinse of sampling tools after taking samples of an *M. hapla* infected rose crop.

Sample	Infection source	Infection level
Roots	Hands	43 J2/sampling point
Substrate	Probe	0,45 J2/sampling point
Nutrient solution	Syringe	0,08 J2/sampling point

Figures

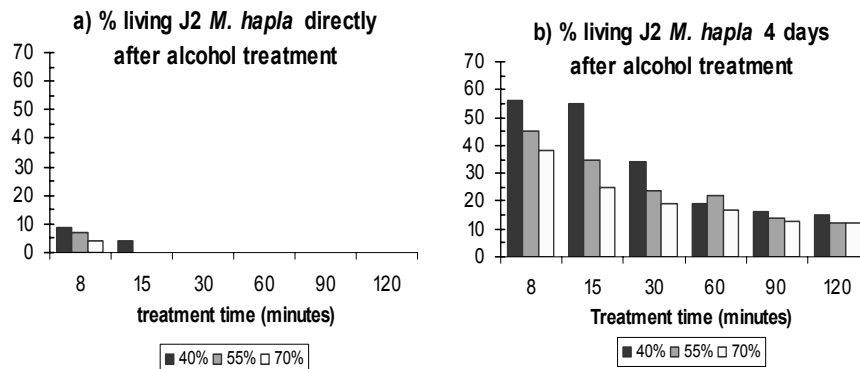


Fig. 1. Effectiveness of ethyl alcohol for tool disinfection against eggs of *M. hapla*, expressed as percentage of living J2 of *M. hapla*: directly a) and four days b) after ethyl alcohol treatment.

