Suitable Rootstocks for Organically Grown Tomato and Cucumber in Relation to *Meloidogyne* spp.

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Abstract

In organic greenhouse vegetable production, significant yield loss occurs due to root-knot nematodes (*Meloidogyne* spp.). Soil steaming is effective; however, it is not preferred, since beneficial soil micro organisms are eliminated. An inclusion of resistant hosts within the crop rotation is complicated by the broad host range of the nematode and the high level of specialization of companies towards production of fruit vegetables. Nearly all species of fruit vegetables are susceptible to a varying degree and complete resistance is absent. Even when rootstocks show partial resistance, this is broken down at high temperatures and with an increase in nematode densities. In addition, a lack of compatibility between the rootstock and graft is reported.

The objective of the research was to find a rootstock with a high resistance against the most important *Meloidogyne* species in greenhouses in the Netherlands, *M. incognita* and *M. hapla*. Secondly, rootstocks should combine resistance with high yield and quality.

In five experiments, 16 to 28 rootstocks per vegetable crop were tested for susceptibility, based on the number and size of root-knots and degree of nematode reproduction. In tomato (*Solanum lycopersicum* L.), one of the best performing rootstocks was 'PG76'; in cucumber (*Cucumis sativus* L.) 'RZ64-10' and 'Harry' performed well. In general, tomato rootstocks have a higher level of resistance against *M. incognita* compared with *M. hapla.* However, cucumber rootstocks seem to be more susceptible to *M. incognita* than to *M. hapla*.

INTRODUCTION

Root knot nematodes (RKN, *Meloidogyne* spp.) cause high economic losses in flower and vegetable production systems worldwide. *Meloidogyne incognita* is considered the most economically important species, as it has a worldwide distribution and a broad host range. Its attributed crop loss is estimated as 5% annually worldwide and it is considered as one as the major obstacles for adequate food supply in many developing countries (Hussey and Janssen, 2002).

In organic greenhouses, the occurrence of root-knot nematodes is one of the most important reasons for significant yield losses in tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.). In Dutch greenhouses with organically grown fruit vegetables, *M. incognita* (*Mi*) and *M. hapla* (*Mh*) are the most common root-knot nematodes, followed by *M. javanica* (*Mj*) (van der Wurff et al., 2010).

Soil steaming is often used as an effective method of control; however, it requires substantial amounts of energy and beneficial soil microorganisms may be eliminated. The use of cultivars or rootstocks with resistance against RKN represents a sustainable control option. Grafting may enhance tolerance to abiotic stress, increase yield, enhance efficient water and nutrient use, extend harvest periods and improve fruit yield and quality (e.g., Lee and Oda, 2003).

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However, nearly all species of fruit vegetables are susceptible to RKN to a varying degree. Even when rootstocks show partial resistance, this is broken down at temperatures above 28°C with increased nematode densities, especially in tomato (Dropkin, 1969; Fassuliotis, 1979). In addition, a lack of compatibility between the rootstock and graft is reported (e.g., Davis et al., 2008).

The objective of this research was to find rootstocks with a high resistance against *Mi* and *Mh*, which also had high yields and quality. The results of several experiments conducted with rootstocks of tomato and cucumber are presented.

MATERIALS AND METHODS

From 2005 to 2009, five experiments were conducted: experiment 1 to 5 with cucumber and experiment 3 to 5 with tomato. In experiments 1 and 2, cucumber rootstocks with 'Aviance' (Rijk Zwaan) were compared with ungrafted 'Aviance'. In experiments 3 and 4, cucumber rootstocks were not grafted. In experiment 5, cucumber rootstocks were grafted with the powdery mildew resistant 'Sudica' and 'Shakira' (both Monsanto) and 'Aviance' (Rijk Zwaan). In experiments 3, 4 and 5 tomato rootstocks were not grafted.

Experiments 1 to 5 started, respectively, in March 2007 and extended fourteen weeks with ten replicates per rootstock; August 2007 for eleven weeks with eight replicates per rootstock, March 2008 for twelve weeks with five replicates per rootstock, August 2008 for ten weeks with five replicates per rootstock and March 2009 for twelve weeks with nine replicates per rootstock. For experiments 1 to 3, $16*10^3$, $9.3*10^3$, $4*10^3$ J2? *Mi* were inoculated per pot, respectively. In experiments 4 and 5, mixed populations of $19.2*10^3$ with *Mi*:*Mh* of 1:1 and $20.3*10^3$ with *Mi*:*Mh*:*Mj* of 64:33:3 were used.

All experiments were performed in a 144 m^2 greenhouse at Bleiswijk, The Netherlands (Longitude 51.989°, Latitude 4.476°), with temperatures ranging from 21.6 to 22.2°C. The minimum temperature was 18°C during the night and maximum temperature was 30°C during the day.

Rootstocks were organically propagated and rooted at Grow Group or Grootscholten, the Netherlands. After approximately 40 days, plantlets were transferred to ten litre pots. Pots contained coarse sand, were sealed with agryl cloth and positioned in rows with about two plants per m^2. Pots were irrigated with a standard nutrient solution with an electrical conductivity (EC) of 1.7 mS cm^{-1}. Stem diameters of plants were measured at a height of 15 cm above the soil.

Second-stage juvenile (J2) cultures were obtained from HZPC Research BV, Metslawier, The Netherlands. Per pot, J2 were added equally spaced around the stem. One day pre-inoculation until 24 h post-inoculation, irrigation was paused to prevent loss of RKN. After 10 to 14 weeks, roots were washed with tap water, dried with paper towels and weighed. Root-knot index (RKI) on a scale of 0 to 10 was recorded for each rootstock, according to van der Wurff et al. (2010). Following this procedure, roots were cut into 3 cm pieces, mixed together, and 50 g fresh weight roots of each rootstock were incubated for four weeks in a mistifier (Seinhorst, 1950) at 20°C. Nematodes were then collected and counted. In experiment 5, RKN were counted and the proportions of *Mi*, *Mh* or *Mj* were estimated subsequently with genus-specific quantitative PCR markers by Blgg AgroXpertus, Wageningen, The Netherlands.

Root-knot index, stem diameter, root weight (RW) data and number of RKN juveniles (J2) extracted from roots were log-transformed, where appropriate, and analyzed using the general linear model (GLM) procedure with a Tukey-test in SPSS 15.0.

RESULTS AND DISCUSSION

Prior to the experiments, an inventory was made of rootstocks with potential resistance against root-knot nematodes. In the first experiments with cucumber (experiment 1 and 2) and tomato (experiment 3), a wide range of rootstocks was tested. In following experiments, the most promising rootstocks were tested again; occasionally, newly obtained rootstocks were added.

Cucumber

In experiment 1, rootstocks 'RZ64-10' and 'Harry' showed a lower RKI compared to the standard 'Aviance' and other rootstocks (Table 1). In subsequent experiments, 'Harry' and 'RZ64-10' showed comparable RKI. In experiment 2, rootstocks with parental species of *Cucurbita maxima* × *C. moschata* showed a higher RKN compared to rootstocks with parental species of *Sycios angulatus* ('Harry') and *Benincasa* ('RZ64-10' and 'RZ64-12').

In experiments 1 and 4, 'RZ64-10' and 'RZ64-12' showed 90% less RKN reproduction when compared with 'Harry' and other rootstocks (Table 1). In experiment 2, reproduction of RKN in 'E88.035' and 'E88.036' was low.

In experiment 5, no interaction was observed between rootstock and scion in RKI ($F_{\text{rootstock x scion}}$ = 1.13, df = 4, P = 0.35) (Table 1). Interestingly, an effect was seen (not shown) of scion on the weight of the rootstock ($F_{\text{rootstock x scion}}$ =5.56, df = 4, P<0.001) irrespective of its identity, i.e., 'Harry', 'RZ64-10' or 'RZ64-12'. Namely, a graft with 'Aviance' and 'Sudica' resulted in a significantly heavier rootstock compared with 'Shakira'.

From experiments 4 and 5, it can be concluded that 'Harry', 'RZ64-10' and 'RZ64-12' were more susceptible to *Mi* compared to *Mh* or *Mj* (Table 2).

Seed production of 'RZ64-10' and 'RZ64-12' (*Benincasa* sp.) was discontinued. At this time, 'Harry' (*Sycios angulatus*) seems to be the only suitable and available rootstock for organic growers. However, 'Harry' has some disadvantages, namely variability in germination, moderate compatibility with scion, susceptibility to rot at the grafting place during growth and, despite the less visible root-knots, ongoing reproduction of RKN and thus an increase in *Meloidogyne* population size in soil.

Tomato

In experiment 3, all rootstocks showed a low RKI, except for 'Vigostar 4411' and 'E28.33458'. In addition, both showed high RKN reproduction rates (Table 3) of *Mi*.

Experiment 4 confirmed that the best rootstocks in experiment 3 have resistance against *Mi* because they showed a low RKI and reproduction of *Mi* compared with the standard 'Mecano'. Cultivar 'Vigostar 4409' showed a low reproduction of *Mh* as well as of *Mi*. Unfortunately, seed production of 'Vigostar 4409' was discontinued; therefore it was not investigated further. Nearly all rootstocks have a higher reproduction rate of *Mh* than of *Mi*, with on average a factor eight difference (Table 3).

In experiment 5, 'PG76' showed a low RKI. In contrast, all rootstocks from Green Seeds appeared to have little or no resistance against *Mi*, *Mh* or *Mj*. Despite the fact that plants were inoculated with twice as many *Mi* as *Mh*, reproduction of *Mh* was, with the exception of the cultivars from Green Seeds, on average ten times higher. A similar result was obtained in experiment 4. Remarkably, the average RKI in experiment 5 was relatively high compared with the two previous experiments; however, the number of offspring was lower when compared with experiment 3.

Of the six rootstocks investigated in experiments 3 to 5, 'Maxifort' and 'Emperador' appeared to have high reproduction of *Meloidogyne* spp., and 'DRO132' and 'PG76' seemed to have relatively low reproduction of *Meloidogyne* spp.

Cultivar 'PG76', and to a lesser extent, 'Brigéor' showed a low RKI. Both tomato rootstocks performed well. Cultivar 'PG76' seems especially promising, since it showed a low RKI, combined with a low RKN reproduction. Similar results were obtained by Cortada et al. (2009).

The standard 'Mecano' and rootstocks from Green Seeds had the thinnest stems and were therefore expected to grow more slowly (Table 4). Generally, it is assumed that root weight and stem thickness are indicators of growth rate.

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Tables

Table 1. Average root-knot index (RKI) and root weight (RW) (g) for cucumber rootstocks in five experiments.

Cultivar	Parental species	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5	
		RKI[7]	RW[8]	RKI	RW	RKI	RW	RKI	RW	RKI	RW
Aviance[1]	*Cucumis sativus*	8.1e[9]	202bc	7.3c	82b						
Azman[1]	*Cucurbita maxima* × *C. moschata*	7.6e	244bc								
Harry[3]	*Sycios angulatus*	3.5b	331c	2.4a	110b	3.3ab	84	3.6	4	2.5a	42ab
TZ148[4]	*Cucurbita maxima* × *C. moschata*			6.1b	67a						
RZ81-07[1]	Unknown	6.6de	199bc								
RZ82-07[1]	Unknown	5.8cd	157ab								
RZ64-10[1]	*Benincasa*	2.0a	93a	3.2a	83b	2.4a	66	3.6	8	2.4a	37a
RZ64-12[1]	*Benincasa*	5.3c	137ab	2.9a	76b	3.8abc	58	3.9	12	3.3b	48b
Adrian[1]	*Cucumis sativus*	7.4e	202bc								
E88.035[2]	*Cucurbita maxima* × *C. moschata*			7.3c	56a						
E88.036[2]	*Cucurbita maxima* × *C. moschata*			6.2b	66a						
WS5299[5]	*Cucurbita maxima* × *C. moschata*			6.2b	87b	6.6c	53				
08-29[1]	Unknown					4.8abc	52				
08-53[1]	Unknown					5.7bc	33				
Becada[1]	Unknown					6.2c	47				
Sakata Kohai Fushinari[6]	*Cucumis sativus*					5.8bc	37				

[1]Rijk Zwaan, [2]Enza seeds, [3]Syngenta, [4]Clause, [5]Uniseeds, [6]Centre for Genetic Resources, The Netherlands.
[7]RKI ranges from 0 to 10, where 0 represents an absence of root-knots and 10 represents all roots with knots, few roots left and plant is dead.
[8]RW= root weight (g).
[9]Letters (abc) indicate significant subgroups as determined with a Tukey's test at P=0.05 on log-transformed data.

Table 2. Average number of root-knot nematodes (RKN) per 50 g roots of cucumber rootstocks in five experiments.

Cultivar	Exp. 1	Exp. 2	Exp. 3	Exp. 4[3]		Exp. 5[3]		
	Mi[1]	*Mi*	*Mi*	*Mi*	*Mh*	*Mi*	*Mh*	*Mj*
Aviance	414500b[2]	41750b						
Azman	459500b							
Harry	415500b	21125b	100561abc	25283	225	71	2	0
TZ148		19125b						
RZ81-07	488500b							
RZ82-07	435000b							
RZ64-10	34000a	22500b	24485a	2235	63	121	0	0
RZ64-12	58500a	35625b	137639abc	2060	12	240	8	0
Adrian	276500b							
E88.035		14250a						
E88.036		15500a						
WS5299		35625b	601550c					
08-29			100639abc					
08-53			655907c					
Becada			266062bc					
Sakata Kohai Fushinari			22646ab					

[1]*Mi* = *Meloidogyne incognita*, *Mh* = *M. hapla*, *Mj* = *M. javanica*.

[2]Letters (abc) indicate significant subgroups as determined with a Tukey-test at P=0.05 on log-transformed data.

[3]Numbers are estimated based on total *Meloidogyne* counts and *Mi*, *Mh* and *Mj* specific quantitative PCR markers.

Table 3. Average root-knot index (RKI) and number of root-knot nematodes (RKN) per 50 g roots of tomato rootstocks in three experiments.

Cultivar	RKI[11]			RKN					
	Exp. 3	Exp. 4	Exp. 5	Exp. 3	Exp. 4		Exp. 5[14]		
	Mi[12]	*Mi*	*Mi*	*Mi*	*Mi*	*Mh*	*Mi*	*Mh*	*Mj*
Maxifort[1]	0.6	0.8ab[13]	5.3cdef	40246abc	1707	993	137	716	0
Multifort[1]	0.6			4000a					
Optifort[1]	0	1.1ab	4.4cde	2330a	714	4201	27	333	0
DRO132[1]	0	1.8ab	4.2cde	2770a	92	1319	8	225	0
DRO136[1]	0			2337a					
Resistar[9]	0	1.5b		5181a	97	2064			
Integro[6]	0			10060abc					
Vigostar 4409[6]	0	0.9ab		25371abc	150	282			
Vigostar 4411[6]	6.0			141201bc					
RS7122[2]	0			31410abc					
RS7123[2]	0	0.8a		23520abc	225	5655			
Emperador[2]	0	1.4ab	3.4bc	3570a	681	4775	77	799	4
Brigéor[7]	0	1.0ab	1.9ab	9292abc	295	5162	23	841	2
PG76[7]	0	0.3a	0.6a	6540ab	172	1814	31	127	0
Titron[10]	0			5509ab					
E28.33197[4]	0			20327abc					
E28.33458[4]	5.2			990760c					
E28.33464[4]	0	0.6b	3.6bcd	22452abc	36	4125	24	243	0
500267[5]	0.2			7710abc					
500294[5]	0.2	1.1ab		5230ab	3	3717			
ST3505[8]	0								
Big Power[2]			2.1ab				34	457	0
No5[3]			6.0ef				5434	1732	189
No7[3]			4.8cdef				3855	2061	155
AN-67[3]			5.0def				4416	4526	156
Tyking 5[3]			4.2cdef				2771	1455	262
DRO138[1]			4.7cdef				103	935	0
Mecano[2]		5.3d	6.4f		5013	883	4977	819	0

[1]Monsanto, [2] Rijk Zwaan, [3]Green Seeds, [4]Enza seeds, [5]Syngenta, [6]Nickerson-Zwaan, [7]Gautier seeds, [8]Uniseed, [9]Hazera, [10]Western Seed.

[11]RKI ranges from 0 to 10, where 0 represents an absence of root-knots and 10 represents all roots with knots, hardly roots left and plant is dead.

[12]*Mi* = *Meloidogyne incognita*, *Mh* = *M. hapla*, *Mj* = *M. javanica*.

[13]Letters (abc) indicate significant subgroups as determined with a Tukey-test at P = 0.05 on log-transformed data.

[14]Numbers are estimated based on total *Meloidogyne* counts and *Mi*, *Mh* and *Mj* specific quantitative PCR markers.

Table 4. Average root weight (RW) (g) and stem diameter of tomato rootstocks in the experiments.

Cultivar	Root weight (g)			Stem diameter (mm)	
	Exp. 3	Exp. 4[2]	Exp. 5	Exp. 4	Exp. 5
Maxifort	80abcd[1]	28	150d	13.7bc	19.5de
Multifort	107bcd				
Optifort	138bcd	25	93cd	13.2abc	18.4de
DRO131	113bcd	15	119d	14.4bc	17.9de
DRO132	76abcd				
Resistar	47ab	29		13.7bc	
Integro	126bcd				
Vigostar4409	32ab	25		12.7ab	
Vigostar4411	40abc				
RS7122	89bcd				
RS7123	156bcd	15		14.0bc	
Emperador	133bcd	29	75cd	14.2c	18.3de
Brigéor	142bcd	29	68abc	13.5bc	14.4cde
PG76	150cd	19	69bcd	13.7bc	16.4cde
Titron	91abcd				
E28.33197	42abc				
E28.33458	219d				
E28.33464	34a	36	110d	11.2a	16.4cde
500267	228d				
500294	174cd	24		13.1ab	
ST3505	64abcd				
Big Power			87cd		15.9bcd
No5			46abc		13.8abc
No7			32a		13.4ab
AN-67			27a		12.4a
Tyking5			30a		13.5abc
DRO138			124d		19.7e
Mecano		26	35ab	12.8abc	13.2a

[1]Letters (abc) indicate significant subgroups as determined with a Tukey's test at P = 0.05 on log-transformed data.
[2]Roots of replicates of the same rootstock were pooled.