

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 of 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 m² 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 m². 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 RKN compared to the standard ‘Aviance’ and other rootstocks (Table 1). In subsequent experiments, ‘Harry’ and ‘RZ64-10’ showed comparable RKN. 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 RKN ($F_{\text{rootstock} \times \text{scion}} = 1.13$, $df = 4$, $P = 0.35$) (Table 1). Interestingly, an effect was seen (not shown) of scion on the weight of the rootstock ($F_{\text{rootstock} \times \text{scion}} = 5.56$, $df = 4$, $P < 0.001$) 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 RKN, 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 RKN 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 RKN. 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 RKN 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 RKN. Both tomato rootstocks performed well. Cultivar ‘PG76’ seems especially promising, since it showed a low RKN, 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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Literature Cited

- Cortada, L., Sorribas, F.J., Ornat, C., Fé Andrés, M. and Verdejo-Lucas, S. 2009. Response of tomato rootstocks carrying the *Mi*-resistance gene to populations of *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. *Eur. J. Plant. Pathol.* 124:337–343.
- Davis, A.R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J.V., Lee, S.-G., Huh, Y.-C., Sun, Z., Miguel, A., King, S.R., Cohen, R. and Lee, J.-M. 2008. Cucurbit grafting. *Critical Reviews in Plant Sciences* 27:50–74.
- Dropkin, V.H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. *Phytopathology* 59:1632–1637.
- Fassuliotis, G. 1979. Plant breeding for root-knot nematode resistance. p.425–453. In: F. Lamberti and C.E. Taylor (eds.), *Root-knot Nematodes (Meloidogyne species). Systematics, Biology and Control*. Academic Press, New York.
- Hussey, R.S. and Janssen, G.J.W. 2002. Root-knot nematodes: *Meloidogyne* species. p.43–70. In: J.L. Starr, R. Cook and J. Bridge (eds.), *Plant Resistance to Parasitic Nematodes*. CAB International, United Kingdom.
- Lee, J.M. and Oda, M. 2003. Grafting of herbaceous vegetables and ornamental crops. *Horticultural Reviews* 28:61–124.
- Seinhorst, J.W. 1950. De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje *Ditylenchus dipsaci* (Kühn) Filipjev) [The importance of the condition of the soil for the occurrence of the incidence of the stem and bulb nematode *Ditylenchus dipsaci*]. *Plantenziekten* 56:289–348. (in Dutch)
- van der Wurff, A.W.G., Janse, J., Kok, C.J. and Zoon, F.C. 2010. Biological control of root knot nematodes in organic vegetable and flower greenhouse cultivation. Report 321. Wageningen UR Greenhouse Horticulture, Bleiswijk. p.64.

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 | <i>Cucumis sativus</i> | 8.1e ⁹ | 202bc | 7.3c | 82b | | | | | | |
| Azman ¹ | <i>Cucurbita maxima</i> × <i>C. moschata</i> | 7.6e | 244bc | | | | | | | | |
| Harry ³ | <i>Sycios angulatus</i> | 3.5b | 331c | 2.4a | 110b | 3.3ab | 84 | 3.6 | 4 | 2.5a | 42ab |
| TZ148 ⁴ | <i>Cucurbita maxima</i> × <i>C. moschata</i> | | | 6.1b | 67a | | | | | | |
| RZ81-07 ¹ | Unknown | 6.6de | 199bc | | | | | | | | |
| RZ82-07 ¹ | Unknown | 5.8cd | 157ab | | | | | | | | |
| RZ64-10 ¹ | <i>Benincasa</i> | 2.0a | 93a | 3.2a | 83b | 2.4a | 66 | 3.6 | 8 | 2.4a | 37a |
| RZ64-12 ¹ | <i>Benincasa</i> | 5.3c | 137ab | 2.9a | 76b | 3.8abc | 58 | 3.9 | 12 | 3.3b | 48b |
| Adrian ¹ | <i>Cucumis sativus</i> | 7.4e | 202bc | | | | | | | | |
| E88.035 ² | <i>Cucurbita maxima</i> × <i>C. moschata</i> | | | 7.3c | 56a | | | | | | |
| E88.036 ² | <i>Cucurbita maxima</i> × <i>C. moschata</i> | | | 6.2b | 66a | | | | | | |
| WS5299 ⁵ | <i>Cucurbita maxima</i> × <i>C. moschata</i> | | | 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 | <i>Cucumis sativus</i> | | | | | 5.8bc | 37 | | | | |

¹Rijk Zwaan, ²Enza seeds, ³Syngenta, ⁴Clause, ⁵Uniseeds, ⁶Centre for Genetic Resources, The Netherlands.

⁷RKI 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.

⁸RW= root weight (g).

⁹Letters (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 | | |
|--------------|------------------------|-----------|-----------|---------------------|-----------|---------------------|-----------|-----------|
| | <i>Mi</i> ¹ | <i>Mi</i> | <i>Mi</i> | <i>Mi</i> | <i>Mh</i> | <i>Mi</i> | <i>Mh</i> | <i>Mj</i> |
| Aviance | 414500b ² | 41750b | | | | | | |
| Azman | 459500b | | | | | | | |
| Harry | 415500b | 21125b | 100561ab | 2528 | | | | |
| TZ148 | | 19125b | c | 3 | 225 | 71 | 2 | 0 |
| RZ81-07 | 488500b | | | | | | | |
| RZ82-07 | 435000b | | | | | | | |
| RZ64-10 | 34000a | 22500b | 24485a | 2235 | 63 | 121 | 0 | 0 |
| RZ64-12 | 58500a | 35625b | 137639ab | | | | | |
| Adrian | 276500b | | c | 2060 | 12 | 240 | 8 | 0 |
| E88.035 | | 14250a | | | | | | |
| E88.036 | | 15500a | | | | | | |
| WS5299 | | 35625b | 601550c | | | | | |
| 08-29 | | | 100639ab | | | | | |
| 08-53 | | | c | | | | | |
| Becada | | | 655907c | | | | | |
| Sakata Kohai | | | 266062bc | | | | | |
| Fushinari | | | 22646ab | | | | | |

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

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

³Numbers 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 | | |
| | <i>Mi</i> ¹² | <i>Mi</i> | <i>Mi</i> | <i>Mi</i> | <i>Mi</i> | <i>Mh</i> | <i>Mi</i> | <i>Mh</i> | <i>Mj</i> |
| 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 |

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

¹¹RKI 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*.

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

¹⁴Numbers 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 |

¹Letters (abc) indicate significant subgroups as determined with a Tukey's test at P = 0.05 on log-transformed data.

²Roots of replicates of the same rootstock were pooled.