

Growth and yield of grafted cucumbers in soil infested with root-knot nematodes

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The aim of this study was to determine the effect of rootstocks on the growth and yield of cucumber (*Cucumis sativus* L.) plants in soils infested with root-knot nematodes (*Meloidogyne* spp.). Cucumber 'Adrian' was grown with its own roots or was grafted onto three rootstocks of *Lagenaria siceraria* (Molina) Standl. ('Emphasis', 'S-1', and 'Gourd'), two interspecific hybrid rootstocks of *Cucurbita maxima* Duchesne × *C. moschata* Duchesne ('Strong Tosa' and 'RS 841 Improved') and zucchini *Cucurbita pepo* L. ('Romanesco Zucchini'). The experiments were conducted in commercial greenhouse, with cucumber grafted onto three rootstocks in the first season and onto six rootstocks in the second spring-summer season. The number of leaves was considerably affected by the rootstock in both seasons, and was the highest for the plants grafted onto interspecific rootstocks (28.0 in the first and 44.9 in the second season). The plants grafted onto 'Strong Tosa' had higher total number of fruits (19.9) and yield (5.38 kg) compared to other rootstocks or non-grafted plants in first season, and the same result was found for two interspecific rootstocks in the second season (6.96 kg and more than 28.9 fruits per plant). The total soluble solids, pH and electrical conductivity of the fruit were not affected by rootstock, while titratable acidity changed with the rootstock type. The grafting of cucumber plants onto different rootstocks was confirmed as an acceptable non-chemical method to compete with the limitations of soils infested with root-knot nematodes, but the effect was highly dependent on the choice of the rootstock.

Key words: *Cucumis sativus*, fruit quality, gall, greenhouse, *Meloidogyne* spp., rootstocks.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most grown crops under protected cultivation systems (greenhouses, etc.) throughout the world; however, despite the expansion of soilless cultivation in greenhouses, there is still considerable acreage of soil-grown cucumbers in older facilities. Due to monoculture or narrow crop rotation in greenhouses, the cucurbit crops are frequently cultivated under unfavorable soil conditions caused by soil-borne diseases and environmental stresses.

Root-knot nematodes (RKN), *Meloidogyne* spp., are obligate endoparasites and are among the most common biotic stressors that can cause serious problems in soil-grown cucurbits (Di Vito et al., 1983; Ploeg and Phillips, 2001). Root-knot nematodes infect roots and cause the production of root galls also known as giant cells (as a result of hypertrophy and frequent cell multiplication).

Plants experience a reduced flow of water and nutrients, and subsequently, reduced vegetative growth and yield. Root-knot nematodes jeopardize the soil-grown greenhouse crops in Croatia (Raspudić et al., 2006) and can cause annual cucumber yield losses of approximately 12% (Main and Gurtz, 1989).

Crop rotation with non-nematode hosts and soil disinfection/fumigation are widely used methods for the prevention and suppression of RKN. However, grafting of tolerant rootstocks, as an alternative to pesticide use, may be a more sustainable approach particularly for organic production. The usage of the RKN-resistant rootstocks has been successful in tomato, but is dependent on the RKN species present, the local RKN population within the species, and on the genetic background of tomato with regard to the *Mi* gene (Lopez-Perez et al., 2006; Cortada et al., 2008; 2009; Verdejo-Lucas et al., 2009; Rivard et al., 2010).

Several species were tested as a potential source of RKN resistance in cucurbits, and varying degrees of resistance were found (Thomason and McKinney, 1959; Fassuliotis and Rau, 1963; Lee and Oda, 2003; Siguenza et al., 2005; Thies and Levi, 2007). However, RKN resistance has not been confirmed in commercial cucurbits (Cohen et al., 2007).

Grafting has numerous effects on plants, and cultivation of grafted plants from the plant family *Cucurbitaceae*

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has been gradually increasing in recent years due to the recognized benefits (Lee et al., 2010). Grafting is often aimed at increasing the abiotic and biotic stress tolerance (Schwarz et al., 2010), but it can also enhance vegetative growth and fruit yield. The benefits of grafting on vegetative growth have been reported for cucurbit-type crops (Goreta et al., 2008; Heidari et al., 2010; Bekhradi et al., 2011).

Grafting onto the interspecific 'RS 841' F₁ hybrid (*Cucurbita maxima* Duchesne × *C. moschata* Duchesne) increased marketable yields of cucumber grown in perlite (Kacjan Maršić and Jakše, 2010). The same rootstock was determined to be the best choice for cucumber due to its high survival rate and high fruit yield (Heidari et al., 2010) and for melon where it also resulted in the highest yield (Crino et al., 2007).

Grafting can increase fruit size (Passam et al., 2005; Pogonyi et al., 2005) and could have a significant impact on the flavor of the fruit and level of phytochemical compounds (Rouphael et al., 2010). An overall increase in titratable acidity and vitamin C content of cucumber fruit was found for plants grafted onto figleaf gourd (*Cucurbita ficifolia* Bouche) and Chaofeng Kangshengwang (*Lagenaria siceraria* (Molina) Standl.), regardless of whether the plants were challenged with saline stress (Huang et al., 2009). Likewise, Proietti et al. (2008) reported higher dehydroascorbate (DAscA) and total vitamin C content for grafted mini-watermelon plants.

Even with chemical treatments, cucumber production has become almost non-profitable in older greenhouses in Croatia; therefore, non-chemical methods should be tested for their effectiveness. Soil solarization was partially effective against RKN (Ioannou, 2001), but it is more effective in the warmest part of the year, during the growing season for cucumber plants. Thus, the aim of this study was to determine the effect of rootstocks on the growth, yield, fruit quality, and root-knot nematodes (RKN) reproduction of cucumbers grown in soils infested with the RKN.

MATERIALS AND METHODS

Experiments

The experiments were conducted in a commercial greenhouse (Trogir, 43°31' N, 16°14' E) with a known history of RKN. The experiments were conducted during two spring-summer growing seasons, with a 1 yr interval between them. Plant materials included cucumber 'Adrian' (Rijk Zwaan, De Lier, The Netherlands) grown with its own roots or grafted onto the rootstocks as follows: three rootstocks of *L. siceraria* 'Emphasis' (Syngenta Seeds SAS, Toulouse, France), 'S-1' (Known-You Seed, Kaohsiung, Taiwan), and Friend (Takii Europe B.V., De Kwakel, The Netherlands); two interspecific hybrid rootstocks of *C. maxima* × *C. moschata* 'Strong Tosa' (Syngenta Seeds) and 'RS 841 Improved' (Royal Sluis,

Enkhuizen, The Netherlands); and squash, *C. pepo* L. 'Romanesco Zucchini' (MIAGRA doo, Zagreb, Croatia). A completely randomized experimental design, with four replicates, was applied. Each treatment consisted of 80 plants for four replicates of 20 plants each.

Seedlings production and plant cultivation

Seedlings were grown in a heated greenhouse as described previously (Goreta et al., 2008), then grafted using the cleft grafting method (Lee and Oda, 2003), and grown under reduced light conditions (10% of daily light intensity) at a relative humidity above 95% and a temperature from 22 to 25 °C until callus formation.

Transplants with four to six fully developed leaves were planted into the soil mulched with polyethylene (PE) semi-transparent brown film (0.02 mm thick and 120 cm width). Drip irrigation tape (T-tape; T-Systems International, San Diego, California, USA) was placed below the black PE film, with emitter spacing at 20 cm (capacity 4 L h⁻¹). Field transplanting was performed on 8 May in the first season and on 15 May in the second season in double strips at a spacing of 50 cm within a row, 60 cm between rows and 90 cm between strips.

Preplant fertilizer, 5N-8.7P-24.9K, was incorporated in the soil at a rate of 500 kg ha⁻¹. Fertigation was applied once every week during the experiment, using a water-soluble fertilizer (Kristalon, Hydro Agri, Rotterdam, The Netherlands). The fertilizer was added at a rate of 120 kg N ha⁻¹, 66 kg P ha⁻¹, and 80 kg K ha⁻¹. Plant protection, weed removal, and training were conducted according to the standard greenhouse practices for cucumber growth in soil. Fruits were harvested every other day from 24 May until 26 July during the first season and from 31 May until 13 July during the second season.

Vegetative growth, yield, and fruit quality measurements

The length of the main stem (from the grafting point to the apex) and the number of leaves (longer than 1 cm) on the main stem were measured once in the first season, 21 d after planting (DAP) and twice in the second season (22 and 43 DAP).

Components of early (first three harvests) and total yields (fruit weight, yield per plant, and number of fruits per plant) were determined for both seasons. Fruit weight, length, and diameter were measured in the middle of harvesting period.

Fruit quality was analyzed in the second season. The total soluble solids (TSS), pH, electrical conductivity (EC), and titratable acidity were measured in the juice obtained by homogenizing the fruit flesh samples in a blender (Moulinex, DPA 443, Ecully Cedex, France) and then filtering out the pureed mesocarp. The TSS was determined using a portable refractometer (DR201, Krüss Optronic GmbH, Hamburg, Germany) and expressed as °Brix. An EC meter (MC 126; Mettler Toledo, Zürich,

Switzerland) and pH meter (MP 120; Mettler Toledo, Zürich, Switzerland) were used for the EC and pH assessments, respectively. Fruit mesocarp acidity was determined by a potentiometric titration with 0.1 M NaOH up to pH 8.1 using 15 mL of the juice and expressed as the concentration (g L⁻¹) of citric acid (AOAC, 1995).

Meloidogyne spp. reproduction assessments

The number of nematodes per gram of roots was determined in the samples collected 76 DAP in the first season and in the samples collected 43 and 70 DAP in the second season. Four plants per treatment and per replicate were pulled from the soil, and their roots were washed and examined. One gram of root per sample (consisting of tissues taken from all parts of the root) was weighed and cut in pieces. The finely chopped root pieces were placed on constructed mesh sieves, and the bottom of the sieve was placed in the water in a grid Petri dish. To extract the nematode from root tissue, samples were incubated for 24 h in water. Then, sieves were removed, and larvae were counted using a stereomicroscope. The *Meloidogyne* spp. identification was confirmed using the key of Jepson (1987). In the second season (43 and 70 DAP), root samples were indexed for galling (on a scale from zero to five, zero indicated no galls, and five indicated > 75% of roots galled; described in Dhandaydham et al., 2008).

Statistical analysis

Data were analyzed using ANOVA with StatView statistical software (StatView for Windows; SAS Institute 1992-1998; Version 5.0). Following an F-test for significance, means were compared using the LSD-test at $P \leq 0.05$.

RESULTS

Vegetative growth

The length of main stem and number of leaves on main stem were affected by the rootstock in both growing seasons (Table 1). In the first season, main stem length and number of leaves were the highest for plants grafted onto 'Strong Tosa'. During the second season, ungrafted plants

had the longest main stem at 22 DAP and were among plants with the highest number of leaves; however, the same effect was not found at 43 DAP. At 43 DAP, 'Strong Tosa' and 'RS 841 Improved' exhibited more vigorous growth than other rootstocks.

Yield and fruit quality

In the first season, early and total yield was higher for plants grafted onto 'Strong Tosa' compared to other rootstocks or ungrafted plants (Table 2). In the second season, early fruit yield was higher for plants grafted onto 'Strong Tosa' and 'RS 841 Improved' rootstocks, than for ungrafted plants and plants grafted onto zucchini. Plants grafted onto 'Strong Tosa' and 'RS 841 Improved' achieved higher total yields than plants grafted onto other rootstocks, or than ungrafted plants.

Plants grafted onto 'Strong Tosa' had the highest early and total number of fruits in the first season (Table 2). However, the number of early fruits was not affected by the rootstock in the second season, whereas 'Strong Tosa' and 'RS 841 Improved' exhibited the highest number of totally harvested fruits.

The fruit weight was not affected by rootstock type in the first season (Table 3). In the second season, the fruit weight was higher for the plants grafted onto 'RS

Table 1. The length of the main stem and number of leaves on the main stem of ungrafted cucumber 'Adrian' plants or scions grafted onto different rootstocks and grown in soil infested with root-knot nematodes during two seasons.

Rootstock	1 st season		2 nd season			
	21 DAP		22 DAP		43 DAP	
	Stem of length	Number of leaves	Stem of length	Number of leaves	Stem of length	Number of leaves
	m		m		m	
Ungrafted	1.67b	26.6b	2.30a	29.7a	3.04c	40.2b
'Emphasis'	1.64b	24.4c	2.12bc	28.0bc	3.07c	41.0b
'S1'	1.70b	25.0c	2.14bc	28.3abc	3.13c	41.0b
'Strong Tosa'	1.87a	28.0a	2.18b	29.6a	3.51a	44.9a
'Friend'	-	-	2.07c	27.3cd	3.19bc	41.3b
'Romanesco Zucchini'	-	-	1.90d	26.4d	2.93c	40.8b
'RS 841 Improved'	-	-	2.18b	28.8ab	3.50ab	44.9a
<i>P</i>	0.001	0.001	0.001	0.001	0.001	0.001

DAP: days after planting.

Mean values (n = 4) followed by different letters in each column indicate significant differences according to LSD test ($P \leq 0.05$).

Table 2. Early and total yield and number of fruits of ungrafted cucumber 'Adrian' plants or scions grafted onto different rootstocks and grown in soil infested with root-knot nematodes during two seasons.

Rootstock	1 st season				2 nd season			
	Early		Total		Early		Total	
	Yield	Number fruit plant ⁻¹						
	kg plant ⁻¹		kg plant ⁻¹		kg plant ⁻¹		kg plant ⁻¹	
Ungrafted	0.59b	2.5b	2.56b	10.1b	0.21b	0.9	4.50b	20.4b
'Emphasis'	0.55b	2.5b	3.03b	11.9b	0.48ab	2.2	4.15b	18.9b
'S1'	0.52b	2.2b	3.07b	11.8b	0.55ab	2.4	4.56b	20.3b
'Strong Tosa'	0.89a	3.9a	5.38a	19.9a	0.61a	2.7	6.96a	29.9a
'Friend'	-	-	-	-	0.55ab	2.4	4.67b	20.9b
'Romanesco Zucchini'	-	-	-	-	0.30b	1.6	4.61b	21.5b
'RS 841 Improved'	-	-	-	-	0.58a	2.7	6.96a	28.9a
<i>P</i>	0.005	0.005	0.001	0.001	0.050	0.090	0.001	0.001

Mean values (n = 4) followed by different letters in each column indicate significant differences according to LSD test ($P \leq 0.05$).

841 Improved' and 'Strong Tosa' than for those grafted onto zucchini 'Emphasis' and 'Friend', or for the ungrafted plants. The fruit length, in the second season, was increased in the scions grafted onto 'Strong Tosa', zucchini and 'Friend' when compared to the scions grafted onto 'Emphasis' or to ungrafted plants (Table 3). However, grafting did not affect fruit diameter.

The total soluble solids, pH, and EC of fruit juice were not affected by rootstock type (Table 4). However, a higher titratable acidity was found in extracts of fruits obtained from the scions grafted onto 'Emphasis' than for those grafted onto 'S1' or for the ungrafted plants.

Nematode reproduction

The number of nematodes per gram of root tissue was not significantly different among tested rootstocks (Table 5). According to the galling index, zucchini roots had fewer galls than roots of the other rootstocks and ungrafted cucumber plants (Table 5). All other rootstocks than zucchini and ungrafted cucumber had 100% root galling on all replicates, indicating very high RKN pressure in the second season. There did not appear to be much RKN pressure in the first season.

DISCUSSION

The cucumber plants were grown in a commercial greenhouse in soil naturally infected with RKN. We evaluated the effect of two interspecific hybrid rootstocks

Table 3. The weight, length and diameter of fruits of ungrafted cucumber 'Adrian' plants or scions grafted onto different rootstocks and grown in soil infested with root-knot nematodes during two seasons.

Rootstock	1 st season		2 nd season	
	Weight	Weight	Length	Diameter
	g	g	cm	mm
Ungrafted	253	220c	18.0b	41
'Emphasis'	252	220c	18.1b	42
'S1'	259	225bc	19.1ab	42
'Strong Tosa'	271	233ab	20.0a	43
'Friend'	-	222c	19.9a	42
'Romanesco Zucchini'	-	215c	20.0a	41
RS 841 Improved	-	236a	19.9ab	43
P	0.084	0.002	0.028	0.767

Mean values (n = 4) followed by different letters in each column indicate significant differences according to LSD test ($P \leq 0.05$).

Table 4. Total soluble solids (TSS), pH, electrical conductivity (EC) and titratable acidity (TA) of fruits of ungrafted cucumber 'Adrian' plants or scions grafted onto different rootstocks and grown in soil infested with root-knot nematodes at 58 d after planting during the second season.

Rootstock	TSS	pH	EC	TA
	°Brix		dS m ⁻¹	g L ⁻¹
Ungrafted	4.54	5.64	3.5	1.03c
'Emphasis'	4.75	5.74	4.3	1.30a
'S1'	4.58	5.70	4.1	1.15bc
'Strong Tosa'	4.16	5.61	4.1	1.18ab
'Friend'	4.46	5.66	3.8	1.17ab
'Romanesco Zucchini'	4.40	5.56	4.3	1.22ab
'RS 841 Improved'	4.68	5.66	4.3	1.07bc
P	0.648	0.225	0.584	0.016

Mean values (n = 4) followed by different letters in the column indicate significant differences according to LSD test ($P \leq 0.05$).

Table 5. Number of nematodes per gram of root and index of galling of ungrafted cucumber 'Adrian' plants or scions grafted onto different rootstocks and grown in soil infested with root-knot nematodes in two seasons.

Rootstock	1 st season	2 nd season			
	Nematodes nr g ⁻¹ root	Nematodes nr g ⁻¹ root		Index of galling [†]	
	76 DAP	43 DAP	70 DAP	43 DAP	70 DAP
Ungrafted	6	37	224	4.3	5.0a
'Emphasis'	3	41	433	4.0	5.0a
'S1'	15	45	343	4.8	5.0a
'Strong Tosa'	7	43	703	3.7	5.0a
'Friend'	-	50	531	3.8	5.0a
'Romanesco Zucchini'	-	80	101	2.5	3.3b
'RS 841 Improved'	-	26	585	3.4	5.0a
P	0.470	0.907	0.560	0.240	0.001

DAP: days after planting.

Mean values (n = 4) followed by different letters in the column indicate significant differences according to LSD test ($P \leq 0.05$).

[†]Galling index scale (0 to 5): 0: no galling, 1: trace infections with a few small galls, 2: < 25% roots galled, 3: 25-50% galling, 4: 50-75% galling, and 5: > 75% of roots galled.

of *C. maxima* × *C. moschata* ('Strong Tosa' and 'RS 841 Improved'), three bottle gourd rootstocks ('Emphasis', 'S-1' and 'Friend') and zucchini ('Romanesco Zucchini') on the vegetative growth, yield, fruit quality, and on RKN reproduction of grafted and ungrafted cucumber plants.

The interspecific hybrid rootstocks enhanced cucumber scion stem length and leaf number, as was also observed by Heidari et al. (2010), whereas the *L. siceraria* rootstocks did not affect scion stem length. In the hydroponic system, cucumber stem length was reported to be unaffected by grafting (Kacjan Maršič and Jakše, 2010).

Fruit weight and length also depended on the choice of the rootstock in our study. Our findings are in agreement with a previous study showing that the 'RS 841 Improved' rootstock produced melon fruits with higher weight compared to *C. melo* rootstock or to ungrafted plants (Crino et al., 2007).

In addition to fruit size, the number of fruit per plant also contributes significantly to yield. Moreover, we observed that the increase in the early and total yield of cucumber plants was obtained by grafting onto the interspecific hybrid rootstocks ('Strong Tosa' and 'RS 841 Improved'). Heidari et al. (2010) found that plants of cucumber grafted onto 'RS 841 Improved' achieved a higher fruit yield in both early and final harvests. Kacjan Maršič and Jakše (2010) also indicated the 'RS 841 Improved' as a rootstock that could provide better marketable yield and fruit number when compared to ungrafted plants. The study of Rivard et al. (2010) was also conducted in a field that was naturally infested with a population of RKN, and showed that tomato fruit yield was higher for plants grafted onto resistant rootstocks. Similarly, Verdejo-Lucas and Sorribas (2008) conducted a study with grafted tomato plants in the soil artificially infested with *Meloidogyne javanica* and reported a higher cumulative yield in the resistant cultivar than in the susceptible control. In addition to resistance, the higher yield of grafted plants could be also a consequence

of increased water and nutrient absorption due to the widespread root system of rootstocks (Salehi et al., 2009).

No differences were observed in TSS (%), pH, and EC from the fruits from plants grafted onto different rootstocks tested in our study. The exception was the titratable acidity that was slightly higher for grafted plants than for ungrafted plants. Davis et al. (2008) noted decreases in TSS and pH of fruits of grafted cucumber, eggplant, and tomato plants.

Reproduction of *Meloidogyne* spp. was observed in two seasons during our study, but a higher reproduction was found in the second season. The same greenhouse was used for tomato and lettuce production between two seasons of our experiment. Lettuce roots were incorporated into the soil at the end of the production cycle, thus we suppose that the infected lettuce roots contributed to the increased density of nematode population during the second season.

Although the number of nematodes ranged from 101 larvae per gram for 'RS 841 Improved' to 703 larvae per gram for 'Strong Tosa' at 70 DAP during the second season, this difference was not significant. A high variability of root infestation was observed within the treatments in our study, which could be explained by an uneven nematode distribution in the soil. Johnson and Boekhoven (1969) observed a significant variability in the population density of *Meloidogyne* spp. in soil samples of 97 cucumber, tomato, and cucumber/tomato greenhouse crops. Spot distribution of symptoms of nematode damage was observed across a vine nursery and was related to the variable population density in the soil (Terlidou, 1974).

The number of *M. javanica* eggs per gram of roots was higher for roots of the ungrafted susceptible tomato than for tested rootstock and standard resistant tomato plants (Verdejo-Lucas and Sorribas, 2008). The final number of nematodes was not lower on roots of grafted melon plants versus ungrafted, therefore, researchers concluded that grafting of susceptible melon scions onto *C. moschata* rootstock made the plants tolerant but not resistant to nematodes (Siguenza et al., 2005). Cucurbit rootstocks that are presently used for grafting of watermelons are highly susceptible to RKN, and other sources of genetic material have been tested (Thies et al., 2010). The rootstocks tested in our study were not resistant to nematode infection and reproduction. However, the robust vegetative growth and higher yields achieved after the grafting onto the 'Strong Tosa' and 'RS 841 Improved' rootstocks may distinguish these rootstocks as more tolerant than the others included in study.

The galling index is usually lower in resistant or tolerant rootstocks than in ungrafted plants (Siguenza et al., 2005; Verdejo-Lucas and Sorribas, 2008; Rivard et al., 2010). The lowest galling index in our study was observed in zucchini roots ('Romanesco Zucchini') during the second season. However, grafting onto zucchini did not promote vegetative growth or yield compared to ungrafted plants. The scions of these grafts were less vigorous, and roots

of zucchini rootstock were less developed (data not shown) and thus the galling index was lower. We suggest that some degree of incompatibility exists between the cucumber scion and zucchini rootstock.

CONCLUSIONS

The vegetative growth and yield of the plants grafted on the interspecific hybrid rootstocks, 'Strong Tosa' and 'RS 841 Improved', were generally the highest despite the high root-knot nematode (RKN) reproduction. Grafting of cucumber plants onto different rootstocks was confirmed as an acceptable non-chemical method to deal with the limitations of soils infected with the RKN; however, the effects were highly dependent on the choice of the rootstock. This method may be especially suitable for small producers that are unable to adopt expensive soilless production systems.

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