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Use of *Cucumis metuliferus* as a Rootstock for Melon to Manage *Meloidogyne incognita*

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Abstract: Root-knot nematode-susceptible melons (Cantaloupe) were grown in pots with varying levels of *Meloidogyne incognita* and were compared to susceptible melons that were grafted onto *Cucumis metuliferus* or *Cucurbita moschata* rootstocks. In addition, the effect of using melons as transplants in nematode-infested soil was compared to direct seeding of melons in nematode-infested soil. There were no differences in shoot or root weight, or severity of root galling between transplanted and direct-seeded non-grafted susceptible melon in nematode-infested soil. Susceptible melon grafted on *C. moschata* rootstocks had lower root gall ratings and, at high nematode densities, higher shoot weights than non-grafted susceptible melons. However, final nematode levels were not lower on the grafted than on the non-grafted plants, and it was therefore concluded that grafting susceptible melon on to *C. moschata* rootstock made the plants tolerant, but not resistant, to the nematodes. Grafting susceptible melons on *C. metuliferus* rootstocks also reduced levels of root galling, prevented shoot weight losses, and resulted in significantly lower nematode levels at harvest. Thus, *C. metuliferus* may be used as a rootstock for melon to prevent both growth reduction and a strong nematode buildup in *M. incognita*-infested soil.

Key words: Cucumis melo, Cucumis metuliferus, Cucurbita moschata, grafting, *Meloidogyne incognita,* melon, reproduction, resistance, rootstock.

The predominant root-knot nematode species infecting melon in California are *Meloidogyne incognita* and *M. javanica*. Both of these species cause dramatic galling on the roots of melon, and very low initial populations can result in considerable yield losses (DiVito et al., 1983; Ferris, 1985; Ploeg and Phillips, 2001). Control of root-knot nematodes and other soilborne problems in melon by soil fumigation with methyl bromide or other nematicides is becoming more difficult because of increased cost of nematicides and legislation banning or limiting their use (Ristaino and Thomas, 1997). As a result, alternative approaches for managing root-knot nematodes in melon are needed. The use of root-knot nematode-resistant varieties has been successful in some crops such as tomato, cotton, and recently peppers and carrot (Ogallo et al., 1997; Roberts, 1992; Simon et al., 2000; Thies et al., 1998). However, root-knot nematode resistance has not been found in *Cucumis melo* (Fassuliotis and Rau, 1963; Thomason and McKinney, 1959). Resistance to root-knot nematodes was found in *C. metuliferus,* but attempts to incorporate this resistance into *C. melo* have not been successful (Chen and Adelberg, 2000; Fassuliotis, 1977; Norton and Granberry, 1980; Soria et al., 1990). One method to circumvent this problem is to graft susceptible scions onto nematode-resistant rootstocks. Successful examples include watermelon or cucumber grafted onto *Sicyos angulatus* (Lee, 1994; Uffelen, 1983); tomato onto *Lycopersicon esculentum, L. pimpinellifolium,* or *L. hirsutum* (Lee, 1994; Renzoni and Lamberti, 1974); and eggplant onto *Solanum torvum* or *L. esculentum* (Ioannou, 2001; Morra, 1998; Porcelli et al., 1990). Grafting of melons onto *Cucurbita* spp. is common in several Mediterranean and Southeast Asian countries but is done mainly to combat *Fusarium* wilt (Lee, 1994). One of the rootstocks (*Cucurbita moschata*) has been used on melon and cucumber because it results in more vigorous plants (Lee, 1994) and may also provide some tolerance against root-knot nematodes (Egelmeers, pers.comm.). Recently, grafting of melons on *Cucurbita* spp. was also shown to be an effective strategy against the sudden wilt disease of melons caused by *Monosporascus cannonbolus* (Edelstein et al., 1999). There are no reports on the use of this approach to minimize root-knot nematode damage in melon. The objective of this study was to evaluate the use of *Curcurbita moschata* and *cucumis metuliferus* as rootstocks for melon to manage *M. incognita.*

Materials and Methods

Nematodes: A race 3 *M. incognita* population, originally isolated from cotton in the San Joaquin Valley, California, was maintained in a greenhouse on tomato var. UC82. Species and race identification were confirmed by isozyme electrophoresis and by reproduction on differential hosts (Eisenback and Triantaphyllou, 1991).

Nematode inocula consisted of *M. incognita* eggs that were extracted from tomato roots with a 1% NaOCl solution in a commercial paint shaker (Radewald et al., 2003). Eggs released from the roots were collected on a 25-µm pore-size sieve and were counted in three 0.1-ml subsamples at 40-fold magnification. Prior to inoculation, egg concentrations were adjusted to contain 10^2, 10^3, 10^4, or 10^5 eggs/15 ml suspension.

Melon grafting: Seeds of *C. metuliferus* PI 292190 (USDA-ARS, Regional Plant Introduction Station, IA), *C. moschata* RZ 64-01 (Rijk-Zwaan, The Netherlands), and melon var. Durango (Seminis, Oxnard, CA) were planted in potting mix in a greenhouse. At the first true-leaf stage, *C. metuliferus* and melon Durango seedlings were cut just below the cotyledons at a 45-degree

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angle. A silicone grafting clip (De Ruiter Seeds Inc., The Netherlands) was slid over the stem of the *C. metuliferus* rootstocks, and the melon scions were then slid on top of the rootstocks, making sure that the 45-degree angle cut surfaces matched. Grafting of melon Durango onto *C. moschata* was done according to the cleft grafting technique (Lee, 1994). The grafted seedlings were then placed in a mist chamber in a greenhouse, delivering a fine mist for 10 secs/min. Seedlings were kept in the mist chamber for 5 days, and the misting interval was changed from 10 sec/min to 10 sec/5 min over this period. Seedlings were then transferred to a greenhouse bench and grown for an additional week prior to transplanting into a 3.5-liter pot.

Experiment setup: Three experiments were conducted. In the first greenhouse experiment the effect of grafting itself was studied. Melon Durango were grafted back on their own rootstock or not grafted. Grafted and non-grafted transplants were planted into 3.5-liter pots containing 0, 2,500, or 25,000 eggs/pot. The experiment had a completely randomized block design, with five replicates for each treatment combination (5 replicates × 2 treatments × 3 nematode densities).

The second experiment was done between May and August in a lath house providing 50% shading. This experiment had two graft treatments: melon Durango grafted onto *C. metuliferus* and melon Durango grafted onto *C. moschata.* These two treatments were compared to Durango melons that were not grafted, and that were seeded directly in the 3.5-liter pots. Nematode densities were 0, 10^2, 10^3, 10^4, or 10^5 eggs/3.5-liter pot. The experiment had a completely randomized block design with six replicates (3 treatments × 5 nematode densities × 6 replicates).

The third experiment, conducted in the greenhouse between January and April, also had three plant treatments: melon Durango grafted onto *C. metuliferus,* non-grafted melon Durango seeded at the same time as *C. metuliferus* and used as transplants, and non-grafted melon Durango seeded in the 3.5-liter pots at the time of transplanting. The experiment was designed as in experiment 2, with six replicates, five nematode densities, and three plant treatments.

For nematode inoculation, 15 ml of egg suspension was thoroughly mixed with 3.4 kg of a 9:1 mixture of steam-sterilized sand and potting mix, and used to fill a 3.5-liter pot. Immediately after filling the pots, the non-grafted, grafted transplants (exps. 1 and 3) and melon seeds (exps. 2 and 3) were added to the pots. Pots were watered through an automated drip system, and 5 days after inoculation 10 g of a slow-release fertilizer (N-P-K: 17-6-10) was added to each pot. Eight weeks after inoculation, plants were carefully removed from the pots. Fresh weights of shoots (including fruits) and roots were determined. Roots were indexed for galling (scale 0-10, 0= no galls, 10= 100% galled) (Bridge and Page, 1980), and eggs were extracted from total root systems by shaking in a 1% NaOCl solution (Radewald et al., 2003) and counted.

Treatment effects were analyzed using ANOVA procedures, and means were separated by Duncan's multiple-range test using SAS software (SAS Institute, Cary, NC). The galling and final nematode data obtained with the no-nematode inoculum density (data all zero) were omitted in the statistical analysis.

Results

Grafting viabilities were 92% for Durango grafted onto their own rootstock in experiment 1; 72% and 79% for melon Durango grafted onto *C. metuliferus* in experiments 2 and 3, respectively; and 89% for melon Durango grafted onto *C. moschata.*

Experiment 1: Grafting melons did not result in differences in the degree of root galling, shoot or root weight compared to non-grafted melons ($P \geq 0.05$) (Table 1). Galling was higher at the highest nematode inoculum ($P \leq 0.05$; data not shown).

Experiment 2: Shoot and root weights, gall rating, and final nematode population levels were different between the three grafting treatments (Table 2). Shoot weight was higher, but root weight, galling, and final nematode populations were lower on *C. metuliferus* rootstocks. Compared to the non-grafted controls, *C. moschata* rootstocks also reduced galling and root weight but did not result in different shoot weights or final nematode populations.

Galling, final nematode populations, and root weight increased with increasing inoculum densities, but total fresh plant weight (root + shoot) was not affected by inoculum level (data not shown). There were also interactive effects between the grafting treatment and the inoculum density for several parameters (Table 2). For example, the shoot weight was much lower at the highest inoculum density, and galling increased significantly with each increase of inoculum density only in non-grafted plants (Table 3).

Experiment 3: No fruits were formed in this experiment because plants were grown in a greenhouse (without pollinating bees). Nematode symptoms were more severe than in experiment 2, with none of the non-grafted melon Durango plants surviving at the highest

Table 1. Average shoot and root weight (g), and gall rating of non-grafted Durango melons and Durango melon grafted back on their own roots.

Treatment	Shoot weight	Root weight	Gall rating[a]
Non-grafted	36.6 a[b]	27.4 a	3.9 a
Grafted	35.5 a	27.2 a	4.1 a

[a] Gall rating on scale from 0 to 10; 0 = no galls, 10 = 100% of root system galled, plant dying.
[b] Different letters within the same column represent significant differences at the 95% confidence level.

TABLE 2. Significance (*P*-value) of treatment effects on melon shoot weight, root weight, gall rating, and *Meloidogyne incognita* levels at harvest (pf).

	P-value			
Treatment factor	Shoot weight	Root weight	Gall rating	Log (Pf+1)
Grafting	0.008	<0.001	<0.001	<0.001
Inoculum density	0.093	<0.001	<0.001	<0.001
Grafting × Inoculum density	0.050	<0.001	<0.001	<0.1446

inoculum density. In contrast, all of the plants grafted onto the *C. metuliferus* rootstocks survived. Grafted plants had higher shoot weights, and lower galling and final nematode populations than non-grafted plants (Table 5). There were no differences between direct-seeded or transplanted non-grafted plants.

However, as in experiment 2, there was an interaction between the plant treatment and the nematode inoculum density on the shoot weight of the melon plants (Table 4) At the highest inoculum density there was a dramatic effect on the non-grafted plants, as none survived. In contrast, all grafted plants survived at this inoculum density, and shoot weights did not differ between the inoculum densities (Table 5).

DISCUSSION

Grafting as a method to control nematodes is common in a variety of perennial fruit crops such as citrus, peach, walnut, grapes, etc. (Brown et al., 1993; Nyczepir and Halbrendt, 1993). Grafting of vegetables, although practiced in some European and Asian countries to control soilborne diseases and to enhance plant vigor (Lee, 1994), has not been widely employed to manage nematode problems. In this study we evaluated grafting of melons, one of the vegetable crops most susceptible to root-knot nematodes (DiVito et al., 1983; Ferris, 1985), onto two rootstocks as an approach to manage *M. incognita*. Results from the first experiment showed that grafting itself did not cause any significant effects on the growth or root gall rating of susceptible melon plants. In the second experiment, we compared the response of non-grafted melons to melons grafted onto *C. moschata* or *C. metuliferus* rootstocks under increasing *M. incognita* pressure. Both of these species have been reported to have increased levels of tolerance or resistance to root-knot nematodes (Egelmeers, pers. comm; Granberry and Norton, 1980; Punja et al., 1988). The total fresh plant weight was not affected by the nematode inoculum level. However, in the non-grafted plants there was a strong shift from shoot to root weight as nematode levels increased. This has been reported previously for melons (Ploeg and Phillips, 2001) and other susceptible plants (Fortnum et al., 1991, 1997; Wallace, 1971). It has been hypothesized that an increased demand of the infested roots for nutrients redirects nutrients away from developing fruits, resulting in fewer fruits developing (McClure, 1977; Ploeg and Phillips, 2001).

Our results showed that susceptible melons grafted to the *C. moschata* rootstock exhibited a high level of tolerance. Galling was reduced, and the average shoot weight was not reduced even at the highest nematode density. However, nematode reproduction on the *C. moschata* roots was high and did not differ from reproduction on the non-grafted controls. Because of this, the *C. moschata* rootstock was omitted in subsequent experiments.

TABLE 3. Effect of *Meloidogyne incognita* inoculum density and rootstock on Durango melon shoot and root weight (g), gall rating, and final nematode populations Pf (eggs/root system).

Parameter Treatment	Inoculum density (eggs/3.5-liter pot)					
Shoot weight	0	100	1,000	10,000	100,000	Mean[a]
Non-grafted	599.3 a[b]	659.5 a	641.2 a	623.8 a	389.3 b	582.6 b[c]
C. moschata	547.6 a	608.8 a	514.3 a	540.0 a	526.0 a	547.3 b
C. metuliferus	611.4 a	642.8 a	634.2 a	656.3 a	671.1 a	643.2 a
Root weight						
Non-grafted	55.4 b	80.9 b	71.0 b	100.5 b	346.5 a	130.9 a
C. moschata	89.5 a	84.1 a	101.1 a	94.1 a	93.8 a	92.5 b
C. metuliferus	36.8 b	31.2 b	33.5 b	31.5 b	55.5 a	37.5 c
Gall rating[d]						
Non-grafted	0	1.3 d	3.0 c	6.5 b	8.0 a	4.7 a
C. moschata	0	0.5 c	1.5 c	2.8 b	6.7 a	2.9 b
C. metuliferus	0	0 c	0.5 c	2.5 b	4.0 a	1.8 c
Pf[e]						
Non-grafted	0	558 d	4,367 c	43,917 b	461,667 a	127,627 a
C. moschata	0	575 d	3,833 c	44,000 b	399,042 a	111,863 a
C. metuliferus	0	267 c	383 b	7,150 a	37,500 a	11,325 b

[a] Data averaged over inoculum density.
[b] Different letters within the same row represent significant differences at the 95% confidence level.
[c] Different letters within the same column (Mean column only) represent significant differences at the 95% confidence level.
[d] Gall rating on scale from 0 to 10; 0 = no galls, 10 = 100% of root system galled, plant dying.
[e] Untransformed data shown, statistical analysis on Log(Pf+1)-transformed data.

TABLE 4. Effect of *Meloidogyne incognita* inoculum density and plant treatment (non-grafted seeded melons, non-grafted transplanted melons, and melon grafted on *C. metuliferus* rootstocks) on melon shoot weight (g), gall rating, and final nematode populations Pf (eggs/root system).

Parameter Treatment	Inoculum density (eggs/3.5-liter pot)					
Shoot weight	0	100	1,000	10,000	100,000	Mean[a]
Non-grafted seed	239.2 a[b]	201.9 a	197.5 a	145.7 a	0.0 b	156.9 b[c]
Non-grafted transplant	269.1 a	251.0 a	195.1 b	57.4 c	0.0 d	154.5 b
C. metuliferus	282.1 a	299.1 a	326.5 a	287.5 a	260.8 a	291.2 a
Gall rating[d]						
Non-grafted seed	0	6.3 b	7.0 b	8.0 b	10.0 a	7.8 a
Non-grafted transplant	0	5.2 d	7.5 c	9.2 b	10.0 a	8.0 a
C. metuliferus	0	1.3 c	2.2 bc	2.8 b	4.7 a	2.8 b
Pf[e]						
Non-grafted seed	0	493,000 a	557,000 a	581,000 a	dead	543,667 a
Non-grafted transplant	0	1,057,083 a	1,620,830 a	530,625 a	dead	1,136,875 a
C. metuliferus	0	1,250 c	10,167 b	49,583 ab	165,042 a	56,510 b

[a] Data averaged over inoculum density.
[b] Different letters within the same row represent significant differences at the 95% confidence level.
[c] Different letters within the same column (Mean column only) represent significant differences at the 95% confidence level.
[d] Gall rating on scale from 0 to 10; 0 = no galls, 10 = 100% of root system galled, plant dying.
[e] Untransformed data shown, statistical analysis on Log(Pf+1)-transformed data.

Previous studies have shown that damage to melons is negatively correlated to the plant age at the time of exposure to root-knot nematodes (Ploeg and Phillips, 2001). In the second experiment, the grafted plants were 4 weeks old at time of exposure to the nematodes, whereas the non-grafted plants were seeded directly in the nematode-inoculated pots. In the third experiment we evaluated whether this difference in the age of the plants may have been responsible for the observed differences in damage and nematode reproduction between the non-grafted and the grafted plants. Four-week-old susceptible non-grafted melons transplanted in nematode-infested soil were compared with susceptible non-grafted melons seeded directly in nematode-infested soil and with 4-week-old melons grafted onto *C. metuliferus*. The results from this experiment showed that both the transplanted and seeded susceptible melons suffered severe nematode damage, with none of the plants surviving at the highest inoculum density. In the second experiment, the plants were grown in an outside lath house where soil temperatures, particularly during the first month, were relatively low (average 20.7 °C). In the third greenhouse experiment average soil temperatures were considerably higher (average 24.7 °C). Activity and reproduction rate of *M. incognita* is favored by soil temperatures of 25 °C to 30 °C (Ploeg and Maris, 1999), and this may explain why plant damage was more severe and final egg numbers were generally higher in the third experiment. Nematode reproduction was not different between the transplanted and seeded non-grafted susceptible melons.

TABLE 5. Significance (*P* - value) of treatment effects on Durango melon shoot weight and gall rating.

Treatment factor	*P* - value	
	Shoot weight	Gall rating
Grafting	<0.001	<0.001
Inoculum density	<0.001	<0.001
Grafting × Inoculum density	<0.001	0.1446

Susceptible Durango melons grafted onto *C. metuliferus* performed well. Under high nematode pressures their shoot weights were significantly higher than the non-grafted plants. In addition, grafting onto *C. metuliferus* rootstocks resulted in a significant reduction in root galling and nematode reproduction. Although nematode reproduction on the *C. metuliferus* roots was lower than on melon roots, *C. metuliferus* rootstocks still allowed significant egg production and should be considered a moderate host for *M. incognita*.

Grafting can be an expensive management tactic. Seeds for both rootstocks and scions need to be purchased, and preparing the grafted plants involves manual labor and careful handling of the grafted transplants (Kurata, 1994; Lee, 1994). In addition, grafting success rates may be well below 100%, making it necessary to graft an excess number of plants. However, progress in the development of grafting robots may decrease dependence on manual labor and may result in lower prices for grafted vegetables (Kurata, 1994). With increasing prices of nematicides and continuing restrictions on their allowed use, grafting may become an economically feasible method in the future.

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