Performance of Grafted Seedless Watermelon Plants with and without Root Excision under Inoculation with *Fusarium oxysporum* f. sp. *niveum* Race 2

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Abstract. Fusarium wilt of watermelon can be effectively managed by grafting with resistant rootstocks. Excision and regeneration of grafted seedling roots is a common practice among cucurbit-grafting nurseries that has not been thoroughly examined. The objectives of this study were to compare the performance of grafted and nongrafted watermelon plants under both greenhouse and field conditions when inoculated with *Fusarium oxysporum* f. sp. *niveum* (FON) race 2, and assess the effect of root excision on growth of grafted plants with *Cucurbita moschata* and *Cucurbita maxima* × *C. moschata* rootstocks. Two greenhouse experiments (Fall 2015 and Spring 2016) and one field trial (Spring 2016) of seedless watermelon ‘Melody’ were conducted in this study. In both greenhouse experiments, inoculated, nongrafted watermelon plants showed a significantly higher percentage of recovered *Fusarium* spp. colonies (70% to 75%) compared with grafted treatments (0% to 7.5%). Some plant growth measurements, including the longest vine length and aboveground fresh and dry weight, indicated less vigorous growth for nongrafted plants compared with the grafted treatments. Significantly higher percent recovery of *Fusarium* spp. below the graft union was observed in the grafted plants with root excision and regeneration treatment (3.7%) in contrast to the intact root treatment (0.5%), suggesting that the root excision method may possibly create entry points for FON infections. Overall, the root excision treatment showed little influence on aboveground growth and root characteristics of grafted plants. Yield of grafted watermelon with FON inoculation in the fumigated field trial was significantly higher than that of noninoculated, nongrafted ‘Melody’ (NGM) control as reflected by the increase of fruit number and size. Averaged over all the grafted treatments, the increase in marketable fruit number and weight reached 108.3% and 240.9%, respectively, and the total fruit number and weight increase was at 80.0% and 237.2%, respectively. However, grafted plants also exhibited greater levels of root-knot nematode infestation as indicated by the significantly higher root galling ratings. Results from this study demonstrated that grafting with squash rootstocks can effectively limit FON colonization in seedless watermelon plants, although more research in rootstock selection and testing is needed to optimize the use of grafted plants for improving plant growth and fruit yield.

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Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is an important specialty crop in Florida, a leading watermelon producer in the United States, with an average production value exceeding \$80 million each year (USDA, 2017). Seedless cultivars are commonly grown by Florida growers, in response to the increasing market demand for seedless watermelon in the United States (Elwakil et al., 2017; Ferreira and Perez, 2016). The tetraploids used in developing triploid watermelons usually have very limited

resistance to fusarium wilt and this may have resulted in most of common seedless watermelon cultivars being susceptible to fusarium wilt (Bruton et al., 2007).

Fusarium wilt of watermelon, caused by FON, is a reemerging pathogen that can cause 100% yield losses in extreme cases (Bruton, 1998). Among the first described fusarium wilt diseases, fusarium wilt of watermelon is still economically important as it occurs worldwide and the pathogen continues to evolve into new and more aggressive races, for which most commercial cultivars lack or have limited resistance (Egel and Martyn, 2013). The phaseout of the broad-spectrum soil fumigant methyl bromide has made it more difficult to manage fusarium wilt (King et al., 2008), thus, requiring producers to use more integrated management strategies including host resistance, biological and chemical controls, crop rotation, and grafting (Everts and Himmelstein, 2015).

Grafting has been widely used in solanaceous and cucurbitaceous crops as a novel integrated disease management strategy, especially when the availability of resistant cultivars is limited. By using selected rootstocks, grafting can efficiently control the soil-borne diseases caused by a wide range of pathogens including nematodes (e.g., root-knot, *Meloidogyne*), fungi (e.g., *Verticillium*, *Fusarium*, *Pyrenochaeta*, and *Monosporascus*), oomycetes (e.g., *Phytophthora*), bacteria (e.g., *Ralstonia*), and several soil-borne viral pathogens (Louws et al., 2010; Thies et al., 2010). Because many commercial watermelon cultivars are susceptible to FON race 2 (Miguel et al., 2004) and race 3 (Egel and Martyn, 2013), interspecific and intergeneric grafting, and the use of interspecific hybrid rootstocks are commonly practiced (Keinath and Hassell, 2014; Louws et al., 2010). Grafting can provide other benefits (e.g., improved fruit yield and lycopene content) besides disease management to watermelon producers, but these benefits can vary depending on the plant material and production systems implemented (Kyriacou et al., 2017; Rouphael et al., 2010).

The vigorous root system from the rootstock can also help improve growth and fruit yield of grafted plants regardless of infections from soil-borne pathogens (Lee et al., 2010). Several studies have confirmed the positive impact of specific rootstocks on plant growth and fruit quality (Alan et al., 2007; Chouka and Jebari, 1997; Kyriacou et al., 2016; Yetisir and Sari, 2003). The use of ‘Shintoza’ (*C. maxima* × *C. moschata*) rootstock increased fruit size and yield stability of grafted plants without affecting fruit quality (Miguel et al., 2004). The interest in watermelon grafting as an effective tool for fusarium wilt control has been identified among growers in Florida; however, to date limited research-based information is available regarding the use of grafted plants in fusarium wilt management in the Florida watermelon production systems.

Depending on grafting skill, available space, and healing environment, different

grafting techniques, including tongue approach, hole insertion, and one-cotyledon grafting, are commonly used for commercial production of grafted watermelon transplants (Davis et al., 2008). In addition, root excision with regeneration of adventitious roots has been used in cucurbit grafting especially when the grafting process is mechanized (Guan and Zhao, 2015). It has also been suggested that a primary nursery can conduct grafting and remove the root system of the grafted plants after healing, while a secondary nursery receiving the shipped grafted plants with root excision can re-root the plants and distribute the re-rooted grafted plants locally (Sabatino, 2013). Root excision could conserve rootstock hypocotyl carbohydrate to improve the healing process (Memmott, 2010). However, it is unclear whether re-rooted, grafted watermelon seedlings will differ from the grafted plants without root excision in terms of their effectiveness in suppressing FON.

It was hypothesized that seedless watermelon plants grafted with selected *C. moschata* and *C. maxima* × *C. moschata* hybrid squash rootstocks could be highly resistant to FON infection and that root excision and regeneration would not affect the performance of grafted plants. Specifically, the objectives of this study were to 1) assess the growth and yield performance of grafted and nongrafted seedless watermelon plants when inoculated with FON race 2 and 2) determine the effect of root excision and regeneration on grafted plant performance under FON race 2 inoculation.

Materials and Methods

Plant materials and grafting. Two greenhouse inoculation experiments and a field inoculation trial were conducted in this study. The seedless watermelon cultivar Melody (*C. lanatus*) (Syngenta® US, Greensboro, NC) was used as the scion in all the experiments. Squash rootstocks ‘Marvel’ (*C. moschata*) (American Takii, Inc., Salinas, CA) and ‘Super Shintosa’ (*C. maxima* × *C. moschata*) (Syngenta® US) were examined in the 2015 greenhouse experiment, whereas an additional *C. maxima* × *C. moschata* rootstock ‘Root Power’ (Sakata Seed America, Morgan Hill, CA) was also included in the 2016 greenhouse experiment. All three rootstocks were tested in the 2016 field experiment with ‘SP-6’ (*C. lanatus*) (Syngenta® US) as the diploid pollenizer. All the transplants used in this study were grown in a greenhouse at the University of Florida campus (Gainesville, FL). The one-cotyledon method was used to graft watermelon seedlings at the first true-leaf stage (Davis et al., 2008).

In the Fall 2015 greenhouse inoculation experiment, ‘Melody’ was sown into 128-cell Styrofoam flats (Speedling, Inc., Ruskin, FL) containing potting mix (Natural & Organic Potting Mix 100%; Sun Gro Horticulture, Agawam, MA) on 29 Sept., and ‘Marvel’ and ‘Super Shintosa’ rootstocks were sown on 30 Sept. and 2 Oct., respectively. Seedlings were

grafted on 8 Oct. Two types of grafted plants were included: plants with intact roots, and plants with root excision and regeneration. Grafted plants with root excision were produced by cutting horizontally at the base of the hypocotyl just above the soil line to completely remove the rootstock roots right after grafting was carried out and inserting the grafted cuttings 2–3 cm deep into a new cell filled with potting mix for development of new adventitious roots around the inserted hypocotyl. The two types of grafted plants were placed into the same healing chamber constructed on a bench in the greenhouse. Graft healing followed the procedure of Liu et al. (2017).

For the 2016 greenhouse inoculation experiment, ‘Melody’ scions were seeded on 11 Apr., and ‘Super Shintosa’, ‘Marvel’, and ‘Root Power’ rootstocks were seeded on 15 Apr. Grafting took place on 22 Apr. with both intact root and root excision treatments. Instead of using the healing chamber placed in the greenhouse, an indoor graft-healing system placed in a walk-in cooler with temperature control was used for the 2016 greenhouse and field experiments (Liu et al., 2017). For the 2016 Spring field inoculation trial, ‘Melody’ watermelon and ‘SP-6’ pollenizer were seeded on 12 Feb., and rootstocks ‘Super Shintosa’, ‘Marvel’, and ‘Root Power’ were seeded on 16 Feb. ‘Melody’ was grafted onto ‘Super Shintosa’ and ‘Marvel’ on 23 Feb., whereas it was grafted onto ‘Root Power’ on 24 Feb.

After the grafted plants were removed from the healing chamber, all the plants including nongrafted and grafted seedlings as well as the pollenizer plants were watered and fertilized daily before field transplanting or planting into the pots as described in Liu et al. 2017.

Greenhouse and field inoculation experiment setup. The greenhouse inoculation experiments were conducted in the greenhouse facilities at the University of Florida, Plant Science Research and Education Unit (PSREU) in Citra, FL, during Fall 2015 and Spring 2016. Both greenhouse experiments were arranged in a randomized complete block design. There was one plant per treatment per block with five blocks in the Fall 2015 experiment, whereas three plants per treatment per block with six blocks were used in the Spring 2016 experiment.

Each grafted plant treatment was transplanted to a 3.79-L black plastic pot filled with potting soil (Natural & Organic Potting Mix 100%; Sun Gro Horticulture). Transplanting took place on 22 Oct. 2015 and 13 May 2016. Preplant fertilizer 10N–4.4P–8.3K with minor elements (Southern States Cooperative, Inc., Richmond, VA) was applied at 15 g per pot on the day before transplanting. Inoculation was conducted after adding fertilizer to the potting soil. Inoculum was produced by inoculating sterile wheat grains at the rate of 10^6 colony-forming units/mL of FON race 2 and incubating it at 25 ± 3 °C for 18–21 d, with a 12-h photoperiod. Flasks with inoculated

wheat grains were shaken daily to promote uniform fungal growth. Plant inoculation was conducted by incorporating the inoculated wheat grains into the potting soil at a concentration of 58 g·m⁻² (0.7 g of infected wheat grains per pot). Inoculated, NGM transplants were used as the control in the Fall 2015 greenhouse study, whereas both noninoculated and inoculated NGM were included as controls in the Spring 2016 greenhouse experiment.

The field experiment was conducted at PSREU in Candler sand soils during Spring 2016. It was arranged in a randomized complete block design with 10 plants per treatment per replication and four replications (blocks). Both noninoculated and inoculated NGM were included as controls. The pollenizers and watermelons were planted at a ratio of 1:3. Plants were grown in raised beds covered with black plastic mulch that were 20 cm high and 76 cm wide, at 2.44 m between-bed spacing and 0.76 m between-plant spacing. The single line drip tape (30.5-cm emitter spacing) was used for irrigation, which was applied daily at 1–2 times and 30–45 min per irrigation event depending on crop growth and developmental stages. Pic Clor-60 (TriEst Ag Group, Inc., Greenville, NC) was applied at the rate of 336 kg·ha⁻¹ for soil fumigation on 1 Mar. Transplanting and inoculation were conducted on 17 Mar. Each planting hole (excluding pollenizer plants) was inoculated with 1.4 g of inoculum before placing the transplants. Preplant application of 10N–4.4P–8.3K fertilizer (Southern States Cooperative, Inc.) was conducted at the rate of 560 kg·ha⁻¹. The 6N–0P–6.6K or 13N–0P–37.4K fertilizer (Mayo Fertilizer, Inc., Lee, FL) was applied through drip irrigation every 7 d during the production season based on crop growth stage and nutrient need (Liu et al., 2017). Throughout the growing season, a standard pest management program was implemented which included preventative applications of fungicides and insecticides following Florida’s watermelon production guidelines (Elwakil et al., 2017; Freeman et al., 2015).

Assessment of plant growth, pathogen recovery, disease severity, root galling, and fruit yield. The greenhouse experiments were ended at 32 and 28 days after transplanting in 2015 and 2016, respectively. Longest vine length, stem diameter (measured at plant crown area above the soil line and below the graft union for grafted plants and at similar height above the soil line for nongrafted plants), and fresh weight of above-ground plant parts were measured at end of the experiments. In addition, aboveground samples were examined for total number of fully expanded leaves and leaf area (measured with LI-3100C Area Meter; LI-COR Inc., Lincoln, NE). Plant roots were washed and root characteristics including total root length, total root surface area, and average root diameter were determined by a root scanner with an image analysis software (WinRhizo 2008a; Regent Instruments, Quebec, QC, Canada). The aboveground biomass

and root dry weight were measured by drying the samples at 60 °C for a week.

Wilt symptoms caused by FON inoculation were not evident in the greenhouse experiments, and thus, pathogen recovery in plant tissue was assessed. The crown including the graft union area was removed from each plant before drying and assessed for the presence of *Fusarium* spp. in the vascular tissue. Eight pieces of stem tissue (≈0.5 cm in length) were cut from each plant, with four pieces collected 1 cm above the graft union and 1 cm below the graft union but above the soil line, respectively. Eight stem tissue samples were taken from the nongrafted watermelon plants in a similar portion of the stem above the soil line. The stem tissue pieces were surface sterilized for 1 min with 10% bleach (containing 5% hypochlorite by weight), rinsed in sterile water, and then placed on potato dextrose agar (20 g of agar per 1 L of deionized water). Plates were stored at 20 ± 2 °C for 3–5 d and then examined for the presence of *Fusarium* spp. using a dissecting scope. *Fusarium* colonies resembling FON including attributes of aerial mycelium color (white or purple) and texture (dense and fluffy) as well as producing curve-shaped conidia (Komada, 1975) were confirmed under the compound microscope and recorded for each plate, and these data were used to indicate the pathogen recovery rate. The stem tissue samples below and above the graft union were not separated for examining FON recovery in the 2015 experiment, so the percent recovery above and below the graft union between the intact root and root excision treatments was only compared in the 2016 experiment by pooling the data across different rootstocks.

The disease severity assessment in the 2016 field trial was conducted starting week 2 (30 Mar.) following field transplanting and

concluded in week 9 (18 May) when a majority of the inoculated nongrafted plants were dead and no new wilting symptoms were observed. *Fusarium* wilt severity was measured based on the estimated percentage of infected canopy area of plants that showed wilting symptoms out of the entire plant canopy in each treatment plot per replication. Three harvests were conducted in the field trial on 8, 21, and 28 June 2016. Both marketable and cull fruit yields were recorded. Ripe fruit (>2.72 kg) without visible defects were harvested from the plots as marketable fruit. Ripe fruit had brown and dry tendrils near the fruit stem end with the bottom of the watermelon (at the soil surface) turning light yellow in color. Cull fruit weighed less than 2.72 kg, were misshapen, had severe hollow heart (Coolong, 2015), and/or had visible damage to the rind and skin. Fruit weight and numbers of marketable and cull fruit were recorded. After the field experiment's final harvest, the plant root systems were removed from the soil and examined for root galling in each plant. Root galls were rated using a 0 to 10 scale (Zeck, 1971): 0 = no galls, 1 = very few small galls, 2 = numerous small galls, 3 = numerous small galls of which some are grown together, 4 = numerous small galls and some big galls, 5 = 25% of roots severely galled, 6 = 50% of roots severely galled, 7 = 75% of roots severely galled, 8 = no healthy roots but plant is still green, 9 = roots rotting and plant dying, and 10 = plant and roots dead.

Statistical analyses. Data analysis was performed using a linear mixed model in the Proc GLIMMIX procedure of SAS program (Version 9.4 for Windows; SAS Institute, Cary, NC). Multiple comparisons of different measurements among the treatments were conducted by Tukey's honestly significant difference test ($\alpha = 0.05$).

Results and Discussion

Greenhouse experiments. In both greenhouse experiments, all the inoculated grafted treatments showed significantly lower average presence (0% to 7.5% recovery) of *Fusarium* spp. in all the crown tissue samples compared with the inoculated, NGM treatment (70% to 75% recovery) (Table 1). This is consistent with previous inoculation studies with races 1 and 2 of FON, which have shown grafted treatments with selected rootstocks to restrict the movement of FON and decrease the presence of fusarium wilt symptoms (Keinath and Hassell, 2014). Our results suggest that the rootstocks can effectively limit FON infections to a level that was almost nondetectable or not significantly different from 0% recovery in the grafted plants.

Fusarium spp. were isolated from some of the grafted plants in both inoculation experiments (Table 1). Nevertheless, the percent recovery was not significantly different from the zero percent recovery observed in other grafted treatments. Some squash rootstocks with resistance to fusarium wilt have been characterized as "asymptomatic hosts" for FON infections (Malcolm et al., 2013). Asymptomatic FON infections of seedless watermelon plants grafted with interspecific hybrid squash rootstocks were reported by Keinath and Hassell (2014). They further pointed out that a scion defense response may be elicited in grafted plants after FON infections occur in resistant rootstocks. A recent study on the resistance of watermelon plants grafted onto the *Lagenaria siceraria* rootstock also shed light on the possible contribution of rootstock exudate composition to suppression of FON conidial germination (Ling et al., 2013).

Table 1. Pathogen recovery, aboveground biomass, longest vine length, stem diameter, total leaf number, and total leaf area in nongrafted and grafted seedless watermelon 'Melody' in 2015 and 2016 greenhouse experiments with *Fusarium oxysporum* f. sp. *niveum* (FON) race 2 inoculation.

Treatment ^c	Pathogen recovery (%) ^y	Aboveground fresh wt (g)	Aboveground dry wt (g)	Longest vine length (cm)	Stem diam (mm)	Total leaf no.	Total leaf area (cm ²)
Fall 2015							
NGM	75.0 a ^x	76.45 c	3.56 c	89.7 c	5.0 c	22	1,270.25 c
M/SS	3.5 b	237.32 ab	16.98 ab	156.0 b	7.1 ab	34	2,801.23 ab
M/SSR	7.5 b	266.00 a	21.97 a	198.2 ab	8.0 a	41	3,334.22 a
M/MV	0 b	176.80 b	11.33 bc	163.4 ab	7.1 ab	32	2,177.73 b
M/MVR	0.4 b	216.07 ab	14.89 b	213.8 a	6.3 b	30	2,908.00 ab
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	<0.01
Spring 2016							
NINGM	2.8 b	388.12 b	84.17 b	230.2 b	6.2 f	105	3,813.16
NGM	70.2 a	334.98 b	78.54 c	192.7 c	7.4 e	92	3,223.32
M/SS	0 b	476.73 a	90.61 a	265.0 a	7.9 de	101	4,347.43
M/SSR	1.3 b	462.48 a	89.84 a	262.8 a	9.1 ab	99	3,865.53
M/MV	0.7 b	469.08 a	91.11 a	273.2 a	8.7 bc	106	4,226.76
M/MVR	5.5 b	458.52 a	92.44 a	274.7 a	9.7 a	99	4,053.59
M/RP	0.7 b	445.06 a	92.56 a	259.3 a	8.4 cd	105	4,035.44
M/RPR	1.3 b	450.52 a	91.22 a	268.6 a	9.2 ab	107	3,900.81
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.59	0.12

^aNGM = nongrafted 'Melody'; NINGM = nongrafted 'Melody' without inoculation; M/SS = 'Melody' grafted onto 'Super Shintosa' with intact root; M/SSR = 'Melody' grafted onto 'Super Shintosa' with root excision; M/MV = 'Melody' grafted onto 'Marvel' with intact root; M/MVR = 'Melody' grafted onto 'Marvel' with root excision; M/RP = 'Melody' grafted onto 'Root Power' with intact root; M/RPR = 'Melody' grafted onto 'Root Power' with root excision.

^yPathogen recovery percentage was calculated by counting the colonies resembling FON including attributes of aerial mycelium color (white or purple), texture (dense and fluffy), and presence of curved conidia, and dividing it by the total number of examined tissue pieces for each plate. All the crown tissue samples above and below the graft union were included in the assessment.

^xMeans within a column within the same experiment followed by the same letter are not significantly different according to Tukey's test at $P \leq 0.05$.

It is also possible for watermelon and the squash rootstock plants to be infected with nonpathogenic *F. oxysporum* and other *Fusarium* spp., which can be present in the potting medium used in this study (Keinath and Hassell, 2014). To address this concern, a noninoculated, NGM control was added in the Spring 2016 greenhouse experiment. A low percent recovery (<3%) of *Fusarium* spp. was also found in this control that was not significantly different from the percent recovery in the grafted treatments (Table 1). Although we did not conduct pathogenicity tests, it was likely that the nonpathogenic *Fusarium* spp. present in the potting soil caused the recovery of *Fusarium* spp. from the noninoculated, NGM plants (Keinath and Hassell, 2014). It was also possible that the *Fusarium* spp. contained in the potting mix might have contributed to the recovery of *Fusarium* spp. from some of the grafted plants in both greenhouse experiments.

There was no significant difference in the recovery of *Fusarium* spp. among the various grafted treatments in the two greenhouse experiments; however, the root excision treatments were on average numerically higher than the nonexcision treatments (Table 1). We did not separate the stem tissue samples for FON recovery in the 2015 experiment, so the percent recovery above and below the graft union between the intact root and root excision treatments was only compared in the 2016 experiment by pooling the data across different rootstocks (Table 2). Significantly higher recovery percentages of *Fusarium* spp. colonies below the graft union were observed in the grafted plants with root excision treatment (3.7%) compared with intact root treatment (0.5%), whereas no significant differences in percent recovery were observed above the graft union ($P = 0.42$). Because the root excision treatment caused wounding on the surface of the rootstock hypocotyl, this result suggests that the root excision method may create entry points for FON infections but that this infection is still limited by grafting with the resistant rootstock. It may also imply that root excision could potentially predispose the plants to other microbial contamination during transplant production and handling. As root excision is being practiced by some grafting nurseries for grafted watermelon transplant production, more research is needed to assess the use of grafted plants with root excision for effective control of fusarium wilt and the impacts of microbial contamination.

In both greenhouse experiments, almost all the grafted treatments significantly increased aboveground fresh and dry weight, and longest vine length as compared with the nongrafted controls including the noninoculated, nongrafted control in the 2016 experiment (Table 1). The grafted plants also showed a significant increase in stem diameter except for plants grafted with ‘Super Shintosa’ without root excision in the 2016 experiment. No significant differences were observed in leaf number among treatments

and controls in either experiment (Table 1). In the 2015 experiment, all the grafted treatments showed significantly greater total leaf area than the nongrafted control. However, nongrafted and grafted plants did not differ significantly in total leaf area in the 2016 experiment ($P = 0.12$; Table 1). Grafted plants also demonstrated a significantly greater total root surface area in the 2015 experiment, whereas no significant difference was observed between grafted and NGM with inoculation in the 2016 experiment (Table 3). The noninoculated, NGM plants showed a significantly lower total root surface area than inoculated plants grafted with ‘Super Shintosa’ and those grafted onto ‘Marvel’ with intact roots in the 2016 experiment. Average root diameter was similar among treatments and controls in both experiments (Table 3). With respect to total root length and root dry weight, inconsistent results were found between the two experiments. Total root length was similar among nongrafted and grafted plants in the 2015 experiment. In the 2016 experiment, all the grafted treatments had significantly higher values of total root length than the nongrafted treatments, without inoculation control, whereas only the grafted treatments with

‘Super Shintosa’ (with root excision) and ‘Marvel’ (without root excision) showed greater values than the inoculated, nongrafted control (Table 3). In addition, similar root dry weight and total root length were observed among nongrafted and grafted plants in the 2015 experiment, whereas plants grafted with ‘Marvel’ and ‘Root Power’ (with root excision) exhibited significantly higher root dry weights than both inoculated and noninoculated, nongrafted controls in the 2016 experiment (Table 3). Considering that the plant growth and root measurements were only conducted at the flowering stage in the greenhouse experiments, it is likely that the advantage of growth enhancement in grafted plants could be more evident as watermelon fruit develop.

The positive impact of grafting on vegetative growth has been consistent with the previous reports, including stem diameter, plant fresh and dry weight, and leaf area (Bekhradi et al., 2011; Huang et al., 2016; Ioannou et al., 2002; Yetisir and Sari, 2003). As indicated in those previous studies, rootstock cultivars had an important effect in determining the vegetative growth performance of the grafted plants. The three rootstocks used in the present study were all

Table 2. Root excision effect on pathogen recovery at the plant base above and below graft union of grafted seedless watermelon ‘Melody’ in 2016 greenhouse experiment with *Fusarium oxysporum* f. sp. *niveum* (FON) race 2 inoculation.

Treatment	Fusarium recovery ^z above graft union (%)	Fusarium recovery below graft union (%)
Intact root	0.5	0.5 b ^y
Root excision	1.8	3.7 a
<i>P</i> value	0.42	0.01

^zFusarium recovery percentage was calculated by counting the colonies resembling FON including attributes of aerial mycelium color (white or purple), texture (dense and fluffy) and presence of curved conidia, and dividing it by the total number of examined tissue pieces for each plate.

^yMeans within a column followed by the same letter are not significantly different according to Tukey’s test at $P \leq 0.05$.

Table 3. Root characteristics of nongrafted and grafted seedless watermelon ‘Melody’ in 2015 and 2016 greenhouse experiments with *Fusarium oxysporum* f. sp. *niveum* race 2 inoculation.

Treatment ^z	Total root surface area (cm ²)	Avg root diam (mm)	Total root length (cm)	Root dry wt (g)
Fall 2015				
NGM	25.67 b ^y	0.49	427.85	0.19
M/SS	30.62 a	0.65	733.19	0.50
M/SSR	33.22 a	0.67	709.65	0.45
M/MV	32.37 a	0.55	620.77	0.29
M/MVR	33.29 a	0.68	673.87	0.43
<i>P</i> value	0.02	0.53	0.31	0.22
Spring 2016				
NINGM	26.99 d	1.31	449.91 c	1.27 c
NGM	29.05 abcd	1.07	510.42 bc	1.18 c
M/SS	31.44 a	1.59	589.53 ab	1.55 abc
M/SSR	30.91 ab	2.07	655.24 a	1.48 bc
M/MV	30.61 abc	1.47	626.27 a	1.94 ab
M/MVR	28.95 bcd	0.53	594.87 ab	2.00 a
M/RP	29.03 bcd	0.52	582.67 ab	1.51 bc
M/RPR	28.47 cd	0.92	587.86 ab	1.92 ab
<i>P</i> value	<0.01	0.28	<0.01	<0.01

^zNGM = nongrafted ‘Melody’; NINGM = nongrafted ‘Melody’ without inoculation; M/SS = ‘Melody’ grafted onto ‘Super Shintosa’ with intact root; M/SSR = ‘Melody’ grafted onto ‘Super Shintosa’ with root excision; M/MV = ‘Melody’ grafted onto ‘Marvel’ with intact root; M/MVR = ‘Melody’ grafted onto ‘Marvel’ with root excision; M/RP = ‘Melody’ grafted onto ‘Root Power’ with intact root; M/RPR = ‘Melody’ grafted onto ‘Root Power’ with root excision.

^yMeans within a column within the same experiment followed by the same letter are not significantly different according to Tukey’s test at $P \leq 0.05$.

squash rootstocks, which could be the reason why most of our results were not significantly different among the grafted treatments. These results indicate that grafting with selected squash rootstocks could possibly promote watermelon plant growth besides limiting FON infections. The influence of grafting with squash rootstocks on root characteristics measured in the two greenhouse experiments tended to be less conclusive. A 3-year field study by Miller et al. (2013) reported that seedless watermelons grafted onto *L. sicaria* and *C. maxima* × *C. moschata* rootstocks did not exhibit differences from nongrafted watermelon plants in terms of root distribution and root length density.

Root excision is a commonly used method for grafted transplants (Guan and Zhao, 2015) and has been indicated to be a beneficial technique (Memmott, 2010). In general, root excision treatments in this study did not affect aboveground growth and root characteristics of grafted plants except for a significant increase in stem diameter in the 2016 experiment (Tables 1 and 3). It has been reported that the quality of grafted transplants with root excision techniques could be affected by healing temperature and duration (Sabatino, 2013). As root excision may be increasingly used by grafting nurseries, future studies are warranted to examine the influence of environmental factors during the healing process, such as temperature and light intensity, on healing quality and growth performance of grafted plants, for optimizing healing of grafted plants with root excision as graft healing and root regeneration take place simultaneously.

Field experiment. On average, the inoculated, NGM control only had a 10% survival rate for all the plants transplanted into the FON-inoculated field plots (Fig. 1), which led to fruit production not significantly different from zero. As a result, NGM was not included in disease, nematode, and yield data analyses. Fusarium wilt disease symptoms were nearly absent in grafted plants and the wilting symptoms were only observed on noninoculated, NGM plants and ‘Melody’ grafted onto ‘Marvel’ with inoculation. The fusarium wilt symptom severity rating was significantly higher in noninoculated, NGM plants (18.8%) than all the grafted treatments (Table 4). The presence of wilting in the noninoculated, nongrafted control plots indicates that natural inoculum of FON was present in the experimental field as no other soil-borne pathogens (e.g., *Pythium*) were noticed in this study. Although the impact of this background inoculum on plant growth and yield was significantly less relative to the artificially inoculated plots, it is important to note that a certain amount of natural inoculum was still present in the fumigated research field. Thus, the results presented here show the effectiveness of using grafted plants for the management of fusarium wilt under high disease pressure compared with nongrafted plants under low disease pressure.

Root-knot nematode (*Meloidogyne incognita*) population densities can also affect the

wilting of watermelon plants in the presence of FON infection (Martyn, 2014). It has been reported that *C. maxima* × *C. moschata* cultivars can be susceptible to root-knot nematodes whereas *C. moschata* cultivars can possess certain levels of tolerance (Huitrón et al., 2007). Similar results were found in our study as the root galling ratings of ‘Melody’ grafted onto the interspecific hybrid squash rootstocks ‘Root Power’ and ‘Super Shintosa’ were significantly higher than that of NGM and ‘Melody’ grafted with the *C. moschata* rootstock ‘Marvel’ (Table 4). Although the nematode infestation level as reflected by the galling rating did not negatively correspond with the plant yield performance in this field study with FON inoculation, integrated management practices and consideration of rootstock susceptibility would be necessary in a producer’s management plan to minimize the risk of yield reduction due to root-knot nematode infestation.

Besides providing fusarium wilt disease control, the grafted plants demonstrated increases in various fruit yield components in this field trial. Despite the rootstock used, all the grafted plants showed a significantly higher average fruit weight than the NGM without inoculation by 68.7% on average, whereas no significant difference was found among the grafted treatments (Table 5). This result is consistent with those reported in previous studies examining the effects of grafting with interspecific hybrid squash rootstocks under field infestation of FON (Keinath and Hassell, 2014; Miguel et al., 2004). The increased fruit size from grafted watermelon may result from the vigorous root systems and the resistance to soil-borne pathogens (Rouphael et al., 2010). Studies on grafted melon (*Cucumis melo*) showed that increased fruit size might also be related to enhanced net photosynthetic rate of leaves as a result of increased leaf area and chlorophyll

Fig. 1. Incidence of fusarium wilt in inoculated plots at the University of Florida Plant Science Research and Education Unit in Citra, FL, 3 weeks after transplanting. The panels show a single replication of the watermelon treatments: nongrafted, inoculated ‘Melody’ (A), nongrafted, noninoculated ‘Melody’ (B), and grafted, inoculated ‘Melody’ scions onto ‘Super Shintosa’ rootstocks (C). Half of the plants observed in panel A are the pollenizer (‘SP-6’). These plots were established in Mar. 2016 and inoculated with *Fusarium oxysporum* f. sp. *niveum* race 2 at transplanting.

Table 4. Fusarium wilt symptom severity, cull fruit yield percentage, and root-knot nematode galling rating of grafted and nongrafted seedless watermelon ‘Melody’ in Spring 2016 field trial with *Fusarium oxysporum* f. sp. *niveum* (FON) race 2 inoculation.

Treatment ^z	Fusarium wilt severity ^y (%)	Cull wt (%)	Cull no. (%)	Root galling ^x
NINGM	18.8 a ^w	8.0	16.5	0.1 c
M/SS	0 b	4.0	6.0	3.5 b
M/MV	2.5 b	4.0	3.8	1.6 c
M/RP	0 b	9.5	11.8	5.2 a
P value	<0.01	0.53	0.21	<0.01

^zNINGM = nongrafted ‘Melody’ without FON inoculation; M/SS = ‘Melody’ grafted onto ‘Super Shintosa’; M/MV = ‘Melody’ grafted onto ‘Marvel’; M/RP = ‘Melody’ grafted onto ‘Root Power’. Data of NGM with inoculation were not available because of the extremely low survival rate in the field following FON inoculation.

^yFusarium wilt severity was measured based on the percentage of infected area of plants that showed wilting symptoms in each plot. The present data were collected on 4 May 2016, 48 d after field inoculation.

^xRoot galling was rated using a 0 to 10 scale: 0 = no galls, 1 = very few small galls, 2 = numerous small galls, 3 = numerous small galls of which some are grown together, 4 = numerous small galls and some big galls, 5 = 25% of roots severely galled, 6 = 50% of roots severely galled, 7 = 75% of roots severely galled, 8 = no healthy roots but plant is still green, 9 = roots rotting and plant dying, and 10 = plant and roots dead (Zeck, 1971).

^wMeans within a column followed by the same letter are not significantly different according to Tukey’s test at $P \leq 0.05$.

Table 5. Average weight per fruit and yield per plant components of grafted and nongrafted seedless watermelon ‘Melody’ in Spring 2016 field trial with *Fusarium oxysporum* f. sp. *niveum* (FON) race 2 inoculation.

Treatment ²	Avg wt/fruit (kg)	Marketable ³ wt/plant (kg)	Marketable no./plant	Total wt/plant (kg)	Total no./plant
NINGM	5.03 b ^x	4.04 d	0.8 d	4.37 d	1.0 d
M/SS	8.08 a	13.12 b	1.7 b	13.69 b	1.8 b
M/MV	8.92 a	10.67 c	1.2 c	11.09 c	1.2 c
M/RP	8.46 a	17.53 a	2.1 a	19.43 a	2.4 a
P value	<0.01	<0.01	<0.01	<0.01	<0.01

²NINGM = nongrafted ‘Melody’ without FON inoculation; M/SS = ‘Melody’ grafted onto ‘Super Shintosa’; M/MV = ‘Melody’ grafted onto ‘Marvel’; M/RP = ‘Melody’ grafted onto ‘Root Power’. Data of NGM with inoculation were not available because of the extremely low survival rate in the field following FON inoculation.

³Ripe fruit (>2.72 kg) without visible defects were harvested from the plots as marketable fruit.

^xMeans within a column followed by the same letter are not significantly different according to Tukey’s test at $P \leq 0.05$.

content (Liu et al., 2011). Although we did not measure leaf area and other growth parameters in the field experiment, our results from the greenhouse experiments suggested the improved total leaf area, root length, and root surface area might be important contributing factors to the increased fruit size of grafted watermelon in the field trial.

In addition to greater average fruit weight, all inoculated, grafted plants had significant increases in marketable and total fruit number and yield weight as compared with non-inoculated NGM (Table 5). Averaged over all the grafted treatments, the increase in marketable fruit number and weight reached 108.3% and 240.9%, respectively, and the total fruit number and weight increase was at 80.0% and 237.2%, respectively. These improved yield results were consistent with other studies on grafted watermelon production (Huitrón et al., 2007; Miguel et al., 2004; Moreno et al., 2016). Stable plant vigor throughout the growing season, improved nutrient and water uptake, and enhanced photosynthesis and carbohydrate metabolism may contribute to the yield increase of the grafted treatments (Huang et al., 2016; Lee et al., 2010; Liu et al., 2011). Moreover, the grafting effects varied significantly among the three rootstocks used with ‘Root Power’ and ‘Marvel’ showing the highest and lowest fruit number and yield, respectively. Interestingly, ‘Root Power’ and ‘Marvel’ also resulted in the highest and lowest root galling ratings, respectively. Furthermore, comparisons between the two interspecific hybrid squash rootstocks demonstrated significantly higher marketable and total fruit yields as well as galling rating in plants grafted with ‘Root Power’ in contrast to plants grafted with ‘Super Shintosa’ (Tables 4 and 5). The root-knot nematode pressure was considered low to intermediate in this trial. The fact that ‘Root Power’ interspecific hybrid squash rootstock was able to maintain high yields under intermediate root-knot nematode pressure and severe fusarium wilt epidemic suggests that more research is needed to assess the dynamics of root-knot nematode infestation in squash rootstocks under various disease situations. The significant differences in root-knot nematode susceptibility and fruit yields of grafted treatments between *C.*

moschata and *C. maxima* × *C. moschata* rootstocks and within the interspecific hybrid rootstocks tested in this study also indicate the feasibility of improving squash rootstocks through selection and breeding to optimize the performance of rootstock-scion combinations taking into consideration the scion genotype, environmental conditions, and production systems.

Conclusions

Both the greenhouse and field experiments with FON inoculation demonstrated the effectiveness of grafting with squash rootstocks for controlling fusarium wilt and improving plant performance in seedless watermelon production. The differential yield performance among different rootstocks in the field suggested that it will be important to take into consideration the susceptibility of interspecific rootstocks to other diseases (e.g., root-knot nematode) and their intrinsic vigor as well as rootstock-scion interactions when selecting them for a region. Excision and regeneration of grafted seedling roots is carried out by cucurbit grafting nurseries; however, our results indicated that this method could potentially predispose the plants to FON infections. More in-depth studies are needed to examine the use of root excision in grafted watermelon transplant production with regard to the impact of the regenerated adventitious root system on disease management and crop growth and yield performance. The results from this study support the use of grafted watermelon plants as an integrated disease management strategy for fusarium wilt (FON race 2); however, continued research is needed to understand how to best employ this strategy in various watermelon production systems.

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