Screening Cucurbit Rootstocks for Resistance to *Meloidogyne* spp. and *Rotylenchulus reniformis*

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Abstract. Fusarium wilt [caused by the fungus *Fusarium oxysporum* f. sp. *niveum* (FON)] has been a consistent problem in watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] production worldwide. One method for combatting this pathogen in the field is to graft a susceptible, high-yielding scion on to a fusarium wilt-resistant rootstock. A concerning issue with rootstocks resistant to fusarium wilt is that they have not been tested for their susceptibility to plant parasitic nematodes—specifically, root-knot nematodes (RKNs; *Meloidogyne* spp.) and the reniform nematode (*Rotylenchulus reniformis*). Preliminary findings have demonstrated that many of these *Fusarium*-resistant rootstocks are highly susceptible to RKNs. Research was conducted during the Spring and Fall 2015 and 2016 to evaluate the resistance to RKN and reniform nematode in rootstocks with known resistance to fusarium wilt. Six rootstocks were evaluated over the course of four experiments. A nematode-susceptible interspecific hybrid [*Cucurbita maxima* (Duchesne) × *C. moschata* (Duchesne)] rootstock ‘Carnivor’ was included as a susceptible control in both years. Results demonstrated that several *Citrullus lanatus* var. *citroides* (L.H. Bailey) rootstocks (‘Carolina Strongback’, USVL246-FR2, USVL252-FR2, and USVL-360) and ‘SP-6’ (a commercially available pollinizer cultivar) exhibited resistance to plant parasitic nematodes when compared with the susceptible control. Partial resistance was observed in USVL-482351. When compared with the control, these rootstocks also had fewer *Meloidogyne* spp. and *R. reniformis* in root tissue. These findings indicate that rootstocks may be available to manage both fusarium wilt and RKN in grafted cucurbit production system.

Though little published data have been collected, it is commonly accepted that damage caused by soil-borne pathogens, such as FON and plant parasitic nematodes (*Meloidogyne* spp.), has increased since the phase-out of methyl bromide. The ban on methyl bromide was estimated to have a significant economic impact, costing consumers and producers at least \$1 billion annually (U.S. Department of Agriculture Economic Research Service, 2000). The loss of methyl bromide has pushed research toward identifying viable alternatives for pathogen management.

A growing method for soil-borne pathogen management in U.S. cucurbit production is grafting. In most cases, regardless of crop, grafting involves the fusion of a high-yielding, susceptible scion to a pathogen-resistant rootstock. Currently, cucurbit grafting is most common in European and Asian countries, where intensive land use and inability to rotate crops have resulted in an increase in soil-borne pathogens. Although resistance to both RKN *Meloidogyne* spp. and FON has been identified independently, no commercial rootstocks or cultivars currently possess resistance to both (Keinath and Hassell, 2014; Pofu et al., 2011; Thies and Levi, 2007; Thies et al., 2010). Research conducted in cantaloupe (*Cucumis melo* L.) demonstrated significant reduction in root gall index (RGI), reproductive factor, and second-stage juveniles (J2) recovered from soil when grafted on RKN-resistant *Cucumis metulifer* (E. Mey. ex Naud.) (Guan et al., 2014). Decreased rates of fusarium wilt incidence and increased yields were observed when watermelon scions were

grafted on fusarium wilt race 2-resistant hybrid rootstocks (Keinath and Hassell, 2014). Previous research shows interspecific (*C. maxima* × *C. moschata*) hybrid rootstocks are susceptible to RKNs and can sometimes perform comparably to susceptible controls (Giné et al., 2017; Guan et al., 2015; Thies et al., 2010). Resistance to both *F. oxysporum* f. sp. *melonis* and *M. incognita* has recently been documented in China in wild cucumber (*Cucumis pustulatus* Naudin ex Hook. f.) (Liu et al. 2014).

Fusarium wilt of watermelon, caused by FON, has four current documented races: 0, 1, 2, and 3. Currently, varietal resistance to races 0 and 1 have been found in commercial watermelon cultivars, but no reliable resistance has been found in races 2 and 3 (Everts and Hochmuth, 2010; Zhou et al., 2010). Fusarium wilt of watermelon, when uncontrolled, has the potential to cause 100% crop loss in a field planted with a susceptible variety (Egel and Martyn, 2007). A major concern with fusarium wilt of watermelon is the longevity and viability of reproductive structures. Chlamydospores formed within the mycelium can persist in soil without a viable host for 15 to 20 years (Egel and Martyn, 2007). Extensive survival in the absence of a host limits the efficacy of crop rotation with a nonhost crop.

Multiple *Meloidogyne* spp. (RKNs) are capable of damaging watermelon (*Citrullus lanatus*), which is susceptible to all four races of *M. incognita*, *M. javanica*, and both races of *M. arenaria* (Sasser and Carter, 1982). Members of the Cucurbitaceae family differ in susceptibility to RKNs, and resistant cultivars are not commercially available (Guan et al., 2014; Levi et al., 2009; Thies and Levi, 2007). It is estimated RKNs cause crop losses of 18% to 33% in subtropical climates annually (Sasser and Carter, 1982). The *M. incognita* damage threshold level of the watermelon cultivar ‘Royal Sweet’ was estimated with an initial population equal to 122 eggs/100 cm³ of soil, 1.6 gal on bioassay roots/100 cm³ of soil, or 3.6 J2/100 cm³ of soil (Xing and Westphal, 2012). *Meloidogyne incognita* is a common pest in sandy soil, which is the dominant component of many soils in Florida.

Historically, *R. reniformis* is not a well-documented pathogen of the family Cucurbitaceae in the United States. In the Southeastern United States, *R. reniformis* is most commonly known as a pest of cotton (Robinson et al., 2005), but has been shown to infest cultivated watermelon (Robinson et al., 1997). In Brazil, *R. reniformis* was shown to reproduce on the roots of cucurbit species, reducing above-ground biomass as nematode populations increase (Torres et al., 2005).

A point of concern arises when both *F. oxysporum* and plant parasitic nematodes occur within the same cropping system, which is not uncommon in sandy soils. A synergistic interaction between pathogens can potentially predispose a resistant host to pathogen susceptibility. Previous literature has demonstrated that when both RKNs and *F. oxysporum* are present in the same area, *Fusarium* symptoms

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increase (Mai and Abawi, 1987; Powell, 1971a, 1971b; Taylor, 1979). However, the literature is vague regarding this interaction, because a disease complex does not always form under these conditions. A study conducted in tomato demonstrated no degradation in host resistance when subjected to a coinoculation of *F. oxysporum* and *M. incognita* (Abawi and Barker, 1984). The primary objective of our experiment was to screen cucurbit rootstocks resistant to FON (Wechter et al., 2016) for additional resistance to RKN and other plant parasitic nematodes.

Materials and Methods

Experiments were conducted during Spring and Fall 2015 and 2016 at the North Florida Research and Education Center located in Quincy, FL. The soil type was Dothan-Fuquay fine, sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiodults-loamy, kaolinitic, thermic Arenic Plinthic Kandiodults). The experiment was arranged as a randomized complete block design with four replications. Soil was cultivated to a depth of 25 cm before bed formation. Rows were spaced 2.44 m apart, and beds were 76.2 cm wide and 20.3 cm tall. The fields used had a history of *R. reniformis* and RKN infestation. Alternative hosts such as yellow squash are planted to maintain nematode populations, but experiments were always conducted after a clean fallow period of one season. White-on-black polyethylene mulch was used in fall, and black polyethylene mulch was used in spring, with irrigation provided by a single off-center drip tape. Seeds were sown in 128-cell transplant trays. Twelve 4-week-old seedlings were transplanted in each experimental plot and spaced 91 cm apart. The rootstock 'Carnivor' (Syngenta Seed, Boise, ID) was used as the fusarium wilt-resistant, RKN-susceptible control. Spring 2015 treatments included 'Carnivor', 'Carolina Strongback' ('CSB') (Wechter, unpublished; Charleston, SC), 'USVL-482351' (Kousik, unpublished), 'USVL246-FR2' (Wechter et al., 2016), 'USVL252-FR2' (Wechter et al., 2016), and 'USVL-360' (Levi, unpublished). Fall 2015 treatments included 'Carnivor', 'CSB', 'USVL-482351', 'USVL246-FR2', and 'USVL252-FR2'. Spring 2016 treatments included 'Carnivor', 'CSB', 'USVL-482351', 'USVL246-FR2', 'USVL252-FR2', and 'SP-6' (Syngenta Seed, Boise, ID), which has resistance to FON 0, 1, and 2. Fall 2016 treatments included 'Carnivor', 'CSB', 'USVL246-FR2', 'USVL252-FR2', 'USVL-360', and 'SP-6'. Rootstock use varied by experiment as a result of limited seed availability on the noncommercial lines.

The RGI is a rating system used to quantify damage caused by RKN on a plant root system. A rating scale of 0 to 10 is used, where 0 indicates no galling and 10 is a completely dead plant (Bridge and Page, 1980). RGI data were collected at 30-day intervals beginning 30 d after planting (DAP) in the Spring and Fall 2016 experiments. Subsequent ratings were collected at

60 DAP for Fall 2015 and Spring and Fall 2016. Final ratings were collected at 90 DAP for all four experiments. Three plants were evaluated at 30 and 60 DAP, and five or all remaining plants were collected and evaluated at 90 DAP. Root systems were excavated and triple-washed before root gall evaluation.

Soil and root tissue samples were collected at 90 DAP for each plot in all but one experiment (Spring 2015). Ten 15-cm soil samples were collected with a hand probe from the individual plots within 2 feet of a plant, then homogenized before collecting a subsample of 100 cc of soil for extraction. For root tissue samples, 10 g of root tissue was collected for each plot and chopped finely to aid in extraction. Nematode extraction from both soil and root tissue was conducted in the same manner. Soil or root samples were first placed in a large coffee filter, then put in a Baermann funnel with water [European and Mediterranean Plant Protection Organization (EPPO), 2013]. After a period of 48 h, nematodes were removed from the bottom of the funnel, and plant parasitic species were counted.

For data analysis, individual plant RGI ratings were first averaged by plot. After plot averages were obtained, the data were subjected to one-way analysis of variance (ANOVA), then mean separation was performed using Fisher's least significant difference (LSD) test at $P = 0.05$ using SAS (version 9.4; SAS Institute, Cary, NC). Nematode soil and root population data were first separated by parasitic species (RKN and *R. reniformis*) to be analyzed independently of one another. Each species was subjected to a one-way ANOVA, with mean separation determined by Fisher's LSD at $P = 0.05$ using SAS.

Results

Spring 2015. 'CSB', and USVL lines '246', '252', and '360' had a significantly lower RGI than 'Carnivor' and 'USVL-482351' at the 90 DAP sampling interval (Table 1).

Fall 2015. 'CSB' and USVL lines '246' and '252' showed resistance in terms of less root galling than 'Carnivor' at both the 60 and 90 DAP sampling intervals (Tables 1 and 2). 'USVL-482351' exhibited a lower RGI at

Table 1. Root gall index evaluated at 90 d postplanting for selected cucurbit rootstocks from research conducted during Spring and Fall 2015 and 2016 in Quincy, FL.

Rootstock	Spring 2015	Fall 2015	Spring 2016	Fall 2016
Carnivor	2.1 a ^z	5.5 a	6.4 a	4.6 a
USVL-482351	1.5 a	1.9 b	1.4 a	np
Carolina Strongback	0.1 b	0.2 c	0.4 b	0.0 b
SP-6	np	np	0.2 b	0.1 b
USVL-246	0.1 b	0.2 c	0.6 b	0.0 b
USVL-252	0.0 b	0.2 c	0.2 b	0.0 b
USVL-360	0.6 b	np	np	0.1 b

^zMeans not followed by the same letter are significantly different at $P = 0.05$ by Fisher's least significant difference. Means were compared within the season.
np = rootstock was not represented in that experiment.

Table 2. Root gall index evaluated at 60 d postplanting for selected cucurbit rootstocks from research conducted during Fall 2015 and Spring and Fall 2016 in Quincy, FL.

Rootstock	Fall 2015	Spring 2016	Fall 2016
Carnivor	3.5 a ^z	3.4 a	2.5 a
USVL-482351	1.7 b	0.6 b	np
Carolina Strongback	0.3 c	0.9 b	0.0 b
SP-6	np	0.6 b	0.0 b
USVL-246	0.4 c	0.3 b	0.0 b
USVL-252	0.5 c	0.1 b	0.0 b
USVL-360	np	np	0.0 b

^zMeans not followed by the same letter are significantly different at $P = 0.05$ by Fisher's least significant difference. Means were compared within the season.
np = rootstock was not represented in that experiment.

Table 3. Nematode populations from soil associated with selected cucurbit rootstocks from research conducted in Quincy, FL, during Fall 2015 and Spring and Fall 2016. *Meloidogyne* spp. S-stage juvenile populations recovered from 100 cm³ soil at 90 d postplanting.

Rootstock	Fall 2015	Spring 2016	Fall 2016
Carnivor	48 b ^z	43 ns	85 ns
USVL-482351	102 a	23	np
Carolina Strongback	2 b	0	51
SP-6	np	6	57
USVL-246	2 b	0	26
USVL-252	0 b	0	14
USVL-360	np	np	43

^zMeans not followed by the same letter are significantly different at $P = 0.05$ by Fisher's least significant difference. Means were compared within season.
np = rootstock was not represented in that experiment; ns = nonsignificant.

both 60 and 90 DAP when compared with the control, but had greater galling than the three previously mentioned lines. Ninety DAP soil populations for both plant parasitic nematode species were found at the greatest density in the 'USVL-482351' rootstock, whereas the control, 'Carnivor', and resistant lines 'CSB', 'USVL246-FR2', and 'USVL252-FR2' had significantly less (Tables 3 and 4). Root tissue populations at 90 DAP for RKN were significantly greater in 'Carnivor' compared with all other lines in the trial (Table 5). However, *R. reniformis* root tissue populations were numerically highest in 'USVL-482351' and similar to the control (Table 6).

Spring 2016. At 60 and 90 DAP RGI, 'Carnivor' exhibited significantly greater root galling than all other lines in this trial. Ninety DAP RKN populations from root tissue were greatest in 'Carnivor', with all other lines having significantly fewer populations. No significance was observed in populations collected from the soil for either species and no significance was found for *R. reniformis* from the root tissue.

During Fall 2016, at both 60 and 90 DAP, the RGI for 'CSB', 'SP-6', and USVL lines '246', '252', and '360' was all significantly less than 'Carnivor' (Tables 1, 2). At 90 DAP, *R. reniformis* soil populations were greater on 'Carnivor' than other entries. Root tissue populations for RKN at 90 DAP were observed to be highest in the control, with similarity to 'SP-6'. 'CSB' and USVL lines '246', '252', and '360' all had fewer RKNs present in the root tissue. *R. reniformis* root tissue populations at 90 DAP were greatest in 'SP-6', with all other experimental entries, including 'Carnivor', having lower populations. No significance was observed at 90 DAP in soil population data for RKN. Soil populations of *R. reniformis* at 90 DAP were greater in 'Carnivor' than all other lines.

Discussion

The primary objective of this study was to screen fusarium wilt-resistant cucurbit rootstocks for resistance to RKN and *R. reniformis*. Throughout all four experiments, the RKN-

susceptible control 'Carnivor' maintained a higher RGI than all treatments, excluding 'USVL-482351' at the Spring 2015 and 2016 90 DAP sampling intervals. Entries 'CSB', 'SP-6', 'USVL246-FR2', and 'USVL252-FR2' consistently exhibited less root galling than 'Carnivor' at both the 60- and 90-DAP sampling intervals in all seasons. Although not always significant, separation also occurred between 'USVL-482351' and other USVL lines. Thirty DAP RGI data were not reported because of minimal (<1.5 on the RGI scale) galling in only two experiments and no significant differences.

Soil populations were not consistently different between rootstocks for RKN. For RKN in the Fall 2015 experiment, the greatest populations were obtained in 'USVL-482351' instead of the control. Soil populations of *R. reniformis* differed among lines in both the Fall 2015 and 2016 experiments. Fall 2015 *R. reniformis* soil data followed a similar trend to the RKN data, with 'USVL-482351' having the greatest density in soil affiliated with the rootstock. In Fall 2016, the highest measured population of *R. reniformis* was on 'Carnivor'. This appears to follow a similar trend in the three experiments in which root tissue was evaluated and in which the RKN-susceptible cultivar Carnivor contained a greater number of RKNs than other lines. Some minor variation occurred during the Fall 2016 trial, when 'SP-6' demonstrated similarity to both the control and other resistant U.S. Department of Agriculture entries. *R. reniformis* root population data were significant in both fall experiments but did not demonstrate any consistent association among treatments. We believe the spatial and overall low presence of *R. reniformis* in the root tissue is largely a result of its feeding habit. *R. reniformis* is a semiendoparasite—meaning, only the anterior body portion is inside the root cortex and the posterior section of its body is exposed (Wang, 2013). The triple-wash procedure performed on roots before collecting root samples may have removed incidentally both sedentary reproductive females and vermiform juveniles.

One of the more interesting results from this experiment is the presence of *R. reniformis* in the root tissue and surrounding soil affiliated with the rootstocks. In the southeastern United States, *R. reniformis* is generally thought of as a major economic pest of cotton or soybeans. The literature is deficient on the economic impact of *R. reniformis* on watermelon and other cucurbit crops. Given the high populations in the soil affiliated with some rootstocks, further research and a possible reconsideration of its pathogenicity on cucurbits should be determined.

This research illustrates there are potential cucurbit rootstocks with resistance to FON and plant parasitic nematodes (PPNs). 'CSB', 'USVL246-FR2', 'USVL252-FR2', 'USVL-360', and 'SP-6' all exhibited resistance to PPNs consistently. 'CSB' and other USVL lines have been bred specifically to serve as cucurbit rootstocks whereas 'SP-6' is a commercially available fusarium wilt-resistant pollenizer for seedless watermelon

Table 4. Nematode populations from soil associated with selected cucurbit rootstocks from research conducted in Quincy, FL, during Fall 2015 and Spring and Fall 2016. Vermiform *R. reniformis* populations recovered from 100 cm³ of soil at 90 d postplanting.

Rootstock	Fall 2015	Spring 2016	Fall 2016
Carnivor	277 b ^z	26 NS	380 a
USVL-482351	628 a	23	np
Carolina Strongback	2 b	0	60 b
SP-6	np	26	87 b
USVL-246	2 b	0	65 b
USVL-252	48 b	0	3 b
USVL-360	np	np	116 b

^zMeans not followed by the same letter are significantly different at $P = 0.05$ by Fisher's least significant difference. Means were compared within the season.

np = rootstock was not represented in that experiment; NS = nonsignificant.

Table 5. Nematode populations from roots of selected cucurbit rootstocks from research conducted in Quincy, FL, during Fall 2015 and Spring and Fall 2016. *Meloidogyne* spp. S-stage juvenile populations recovered from 10 g root tissue at 90 d postplanting.

Rootstock	Fall 2015	Spring 2016	Fall 2016
Carnivor	209 a ^z	88 a	119 a
USVL-482351	102 b	28 b	np
Carolina Strongback	0 b	0 b	14 b
SP-6	np	28 b	42 ab
USVL-246	2 b	9 b	0 b
USVL-252	0 b	3 b	3 b
USVL-360	np	np	3 b

^zMeans not followed by the same letter are significantly different at $P = 0.05$ by Fisher's least significant difference. Means were compared within season.

np = rootstock was not represented in that experiment.

Table 6. Nematode populations from roots of selected cucurbit rootstocks from research conducted in Quincy, FL, during Fall 2015 and Spring and Fall 2016. Vermiform *R. reniformis* populations recovered from 10 g root tissue at 90 d postplanting.

Rootstock	Fall 2015	Spring 2016	Fall 2016
Carnivor	41 ab ^z	0 NS	0 b
USVL-482351	52 a	0	np
Carolina Strongback	0 b	0	0 b
SP-6	np	0	30 a
USVL-246	0 b	0	3 b
USVL-252	0 b	0	0 b
USVL-360	np	np	0 b

^zMeans not followed by the same letter are significantly different at $P = 0.05$ by Fisher's least significant difference. Means were compared within the season.

np = rootstock was not represented in that experiment; NS = nonsignificant.

production. ‘SP-6’ is not currently used as a rootstock, but resistance to FON 0, 1, and 2 demonstrated in this experiment to RKNs makes it a viable candidate for rootstock use when both FON and RKNs are present in a field.

Grafting has the potential to be an additional management tool for tolerance to multiple soil-borne diseases. The availability of cucurbit rootstocks that have resistance to FON and PPNs is especially advantageous because as the two pathogens are often found together. The use of resistant grafted plants also can reduce the need to manage PPNs in grafted watermelon, which is currently a concern as a result of the nematode susceptibility of interspecific hybrid squash rootstocks.

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