Control of Fusarium Wilt of Watermelon by Grafting onto Bottlegourd or Interspecific Hybrid Squash Despite Colonization of Rootstocks by *Fusarium*

A. P. Keinath and R. L. Hassell, Clemson University, Coastal Research and Education Center, Charleston, SC 29414

Abstract

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Grafting watermelon (*Citrullus lanatus* var. *lanatus*) onto rootstocks of interspecific hybrid squash (*Cucurbita moschata* × *C. maxima*), bottle gourd (*Lagenaria siceraria*), or citron (*Citrullus lanatus* var. *citroides*) has been used in Asia and Israel to manage Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum*. The objectives of this study were to determine the frequency of infection of six rootstocks by *F. oxysporum* f. sp. *niveum* races 1 and 2 and the field performance of grafted rootstocks in Charleston, SC. Grafted and nongrafted watermelon and rootstock plants were inoculated in the greenhouse with race 1, race 2, or water (the control treatment). With both races, the frequency of recovery of *F. oxysporum* from scion and rootstock portions of inoculated watermelon plants grafted onto 'Ojakkyo' citron was greater than from watermelon plants grafted onto 'Shintosa Camel' and 'Strong Tosa' interspecific hybrid squash, and from plants grafted onto 'Emphasis', 'Macis', and 'WMXP 3945' bottlegourd. For nongrafted plants inoculated with race 1, percent recovery also was greater from Ojakkyo than from interspecific hybrid squash and bot-

tlegourd. For nongrafted plants inoculated with race 2, *F. oxysporum* was recovered from the base of ≥79% of all inoculated plants. More than two-thirds (15) of 21 isolates recovered from the tops or scions of inoculated plants were pathogenic on watermelon. In spring 2010 and 2011, the six rootstocks were grafted with seedless watermelon 'Tri-X 313', which is susceptible to both races, and transplanted in a field infested with races 1 and 2 of *F. oxysporum* f. sp. *niveum*. Disease incidence for nongrafted and self-grafted Tri-X 313 (the control treatments) and Tri-X 313 grafted onto Ojakkyo citron did not differ significantly. Grafted watermelon plants produced greater weights and numbers of fruit than plants of the two control treatments. Nonpathogenic isolates of *F. oxysporum* and isolates of *F. oxysporum* f. sp. *niveum* colonized interspecific hybrid squash, bottlegourd, and grafted watermelon. The rootstocks evaluated, however, restricted movement of *F. oxysporum* f. sp. *niveum* into the watermelon scion, suppressed wilt symptoms, and increased fruit yields in an infested field.

Fusarium wilt is a widespread, damaging disease of watermelon (*Citrullus lanatus* var. *lanatus*) throughout the United States, Turkey, and other watermelon-producing countries (26,44,45). Cropping the same fields to watermelon without adequate rotation to other crops or use of soil fumigants selects for and increases the population density of the specialized form of the soilborne fungus *Fusarium oxysporum*, *F. oxysporum* f. sp. *niveum*, that infects watermelon (12,32). Fusarium wilt results in the death of individual vines or whole plants, directly reducing the number of fruit produced (15). In addition, the pathogen persists indefinitely in infested soil (30,36). The four races of *F. oxysporum* f. sp. *niveum*—races 0, 1, 2, and 3—are identified by virulence on differential watermelon cultivars (29,49). Race 0 is pathogenic only on older or heirloom watermelon cultivars that have no resistance. Most hybrid, diploid, seeded cultivars have resistance to race 1. Race 2 is present in all major watermelon-producing states in the United States (18). In 2010, race 3 was found in Maryland (49).

Traditionally, 7- to 12-year rotations have been recommended to manage Fusarium wilt (30,36). Many watermelon growers specialize in watermelon production and cannot leave land out of produc-

tion for more than 1 or 2 years (47). In South Carolina, because all seedless watermelon and a majority of seeded watermelon crops are irrigated, growers are limited to planting in fields equipped with wells, pumps, or center-pivot irrigation. Although diploid, seeded watermelon cultivars resistant to race 1 were used in the past to manage this disease, the watermelon market has shifted to seedless fruit (R. L. Hassell, unpublished data). Most seedless, triploid cultivars are susceptible to Fusarium wilt caused by *F. oxysporum* f. sp. *niveum* race 1, and all diploid and triploid watermelon cultivars are susceptible to *F. oxysporum* f. sp. *niveum* races 2 and 3 (18). Soil fumigants, such as methyl bromide applied with various percentages of chloropicrin and 1,3-dichloropropene plus 35% chloropicrin, have been used to manage Fusarium wilt with varying degrees of success (15,35). Additional worker protection and environmental safety requirements for the use of soil fumigants initiated by the United States Environmental Protection Agency in 2010 have made use of these pesticides more costly and time-consuming (41).

Grafting watermelon is done in various countries, notably Korea, Japan, and Israel, to combat soilborne pathogens and increase yield (3,22,26,27). The two most common rootstocks used for grafting watermelon are cultivars of an interspecific hybrid squash (*Cucurbita moschata* × *C. maxima*) and cultivars of bottle gourd (*Lagenaria siceraria*) that were bred specifically for use as rootstocks (19,43). Citron (*Citrullus lanatus* var. *citroides*) is being tested as another rootstock because some genotypes have resistance to root-knot nematode (39). Because *F. oxysporum* f. sp. *niveum* was defined as host-specific to watermelon, the fungus does not cause Fusarium wilt on other cucurbits (30,49). Resistance to Fusarium wilt in cucurbit rootstocks should prevent Fusarium wilt in the watermelon scion. However, *F. oxysporum* f. sp. *niveum* has been reported to infect a few other cucurbits, including seedlings and young plants of several summer squash (*Cucurbita pepo*) cultivars (31). In Tunisia, several cultivars of interspecific hybrid squash used as rootstocks were susceptible (i.e., had >30% wilted plants)

Corresponding author: A. P. Keinath, E-mail: tknth@clemson.edu

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when inoculated in the greenhouse with Tunisian isolates of *F. oxysporum* f. sp. *niveum* (1). Thus, it is possible that *F. oxysporum* f. sp. *niveum* could infect rootstocks and move into the scion, causing symptoms of Fusarium wilt. The frequency of such scion colonization is unknown but should be determined to assess the potential effectiveness of grafting watermelon onto cucurbit rootstocks to manage Fusarium wilt. The objectives of this study were to (i) evaluate infection by *F. oxysporum* f. sp. *niveum* of different genera of cucurbit rootstocks and of watermelon grafted onto these rootstocks and (ii) evaluate control of Fusarium wilt in a field naturally infested with *F. oxysporum* f. sp. *niveum* using watermelon grafted onto cucurbit rootstocks.

Materials and Methods

Rootstocks and grafting procedure. Six rootstocks were grafted with seedless watermelon Tri-X 313 (Syngenta Seeds; Table 1). All seed were sown in 72-cell plug trays with cell depth of 5.7 cm, cell bottom diameter of 2.5 cm, and cell top diameter of 4.0 cm (TLC Polyform) filled with a fertilizer-free, soilless mix (76% sphagnum peat and 25% perlite) provided by Sun Gro Horticulture. No fertilizer was applied during the experiments. Seeding of the different cultivars was staggered to ensure that the hypocotyls of all varieties were the appropriate diameter (3 mm) when grafted, and rootstocks were at the appropriate developmental stage (tip of the first true leaf just beginning to emerge) to minimize regrowth of the tops of rootstocks (11). Watermelon, used as the scion, was seeded first. Flats were placed on a heating pad set at 32°C, covered with black polyethylene film for 48 h to induce germination, and then removed from the heating pad and placed in a greenhouse at 24°C. When the cotyledons of the scion material had fully expanded, the bottlegourd rootstock cultivars and ‘Ojakkyo’ were seeded and incubated in the greenhouse. When the bottlegourd seedlings had emerged (usually 3 days after seeding), the interspecific squash hybrid cultivars were seeded.

Approximately 10 days after seeding the squash, when the watermelon scion was at the first true-leaf stage, the watermelon was grafted onto the rootstocks using the one-cotyledon grafting method (11). Grafted plants were maintained in a healing chamber with 90% humidity at 24°C for 7 days, then grown on a greenhouse bench for an additional 7 days. In order to determine whether the grafting procedure, which included wounding and healing phases, had an effect on infection of the scion by *F. oxysporum* f. sp. *niveum*, a self-grafted control treatment was included in the second experiment, with inoculations using both races of the pathogen, in which Tri-X 313 seedlings were used as both the scion and the rootstock.

Greenhouse inoculations. Grafted and nongrafted plants were inoculated in the greenhouse 14 days after grafting with an isolate of *F. oxysporum* f. sp. *niveum* race 1 or an isolate of race 2. In total, eight separate inoculation experiments were done: four experiments with nongrafted plants and four experiments with rootstocks grafted with seedless watermelon. Of the eight experiments, four were with race 1 and four with race 2. Details are presented below.

F. oxysporum f. sp. *niveum* isolate B05-07, which was identified previously as race 1, and isolate B05-30, which was identified previously as race 2, originated from naturally infected mini-water-

melon cultivars grown at the Clemson University Coastal Research and Education Center (CREC) (32°47′30.4 N, 80°4′11.16 W) in 2005 (14,16). To prepare inoculum, isolates were grown on quarter-strength potato dextrose agar (PDA) for 7 to 10 days. Three 1-cm² pieces of agar with mycelium of the appropriate isolate were added to 100 ml of potato dextrose broth (PDB), and grown on a shaker table at 125 rpm for 7 days at 24°C. The broth was filtered through two layers of sterile cheesecloth. Spores were counted on a hemocytometer and the concentration was adjusted to 10⁶ microconidia/ml.

To inoculate grafted plants, the roots were rinsed gently to remove most of the adhering potting medium and dipped for 10 s in the microconidial suspension. Each plant was transplanted into a separate 7.5-cm-diameter pot (8.5 cm tall) in Fafard 3B potting soil. The experimental design was a randomized complete block with 20 replications of individual plants; each plant served as an experimental unit. Nongrafted ‘Allsweet’ watermelon, resistant to *F. oxysporum* f. sp. *niveum* race 1 and susceptible to race 2, and Tri-X 313, susceptible to both races, were included as inoculated control treatments (45). In the second experiments with both races, self-grafted Tri-X 313 was included as a grafting control treatment. Roots of noninoculated control plants were dipped in water for 10 s. In the first experiments with both races, 10 noninoculated plants of each cultivar were used and, in the second experiments, 20 noninoculated plants per cultivar were used. Plants were maintained in the greenhouse at 24 to 27°C for 3 weeks. Disease incidence based on symptoms of wilting was recorded 14 and 21 days after inoculation.

In separate experiments, nongrafted plants of the same cultivars were inoculated with both races of *F. oxysporum* f. sp. *niveum*. In these experiments, four plants per pot were transplanted, with five replicate pots, for a total of 20 inoculated and 20 noninoculated plants per cultivar. The cultivars were seeded in the same sequence as the plants used for grafting, inoculated using the same procedure described above, and evaluated 14 and 21 days after inoculation. Allsweet and Tri-X 313 were included as control treatments, except that, in the first experiment with race 2, the diploid susceptible ‘Black Diamond’ was used in place of Tri-X 313 (Table 1).

At the termination of each experiment 21 days after inoculation, two 0.5-cm-diameter pieces were cut from the base of the main stem of each plant starting approximately 1 cm above the soil line, and two pieces of the stem were cut from between the first and second true leaf of each plant. Thus, on grafted plants, the base of the rootstock and the top of the watermelon scion were sampled whereas, on nongrafted plants, pieces were cut from the base and top of the same plant. In this context, “scion” refers to the top of a grafted plant and “top” refers to the top of a nongrafted plant. Pieces were surface disinfested for 30 s with 0.5% sodium hypochlorite, rinsed in sterile water, and placed on Komada’s agar medium to determine whether *F. oxysporum* was present in the piece (20). Plates were held at 20 to 22°C with 16-h day length. After 8 to 9 days, colonies resembling *F. oxysporum* were counted based on color (white to pale orange or salmon) and texture (dense and fluffy) of aerial mycelium and abaxial colony color (dull purple to purple-brown) (20). The top or base of the plant was considered infected if one or both of the pieces yielded a colony that resembled *F. oxysporum*.

Table 1. Cultivars of watermelon and cucurbit rootstocks used in this study in Charleston, SC to evaluate for control of Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum*

Cultivar	Species	Common name	Source	Abbreviation
Tri-X 313	<i>Citrullus lanatus</i> var. <i>lanatus</i>	Seedless, triploid watermelon	Syngenta Seeds, Boise, ID	TX
Allsweet	<i>C. lanatus</i> var. <i>lanatus</i>	Seeded, diploid watermelon	Everwilde Farms, Sand Creek, WI	AS
Black Diamond	<i>C. lanatus</i> var. <i>lanatus</i>	Seeded, diploid watermelon	Everwilde Farms, Sand Creek, WI	BD
Emphasis	<i>Lagenaria siceraria</i>	Bottlegourd	Syngenta Seeds, Boise, ID	EM
Macis	<i>L. siceraria</i>	Bottlegourd	Nunhems USA, Parma, ID	MA
WMXP 3945	<i>L. siceraria</i>	Bottlegourd	Harris Moran Seed Co., Modesto, CA	WX
Shintosa Camel	<i>Cucurbita moschata</i> × <i>C. maxima</i>	Interspecific hybrid squash	Nunhems USA, Parma, ID	SC
Strong Tosa	<i>C. moschata</i> × <i>C. maxima</i>	Interspecific hybrid squash	Syngenta Seeds, Boise, ID	ST
Ojakkyo	<i>Citrullus lanatus</i> var. <i>citroides</i>	Citron	Syngenta Seeds, Boise, ID	OJ

Pathogenicity tests. Isolates of *F. oxysporum* recovered from noninoculated plants (73 isolates) or inoculated plants (30 isolates) were checked for pathogenicity to seedlings of 'Sugar Baby' watermelon, which is very susceptible to all races of *F. oxysporum* f. sp. *niveum* (45). Between 3 and 24 isolates were saved from each experiment for pathogenicity testing. The rapid pathogenicity assay described by Zhou and Everts (45) was used, except that three seeds in each of two cells of a 128-cell tray were inoculated with each isolate instead of seeds in one cell. In all tests, the race 1 and 2 isolates used in the inoculation experiments were included as control treatments. Noninoculated seed were treated similarly with water. At 14 days after inoculation and seeding, isolates were considered pathogenic if at least one seedling in each inoculated cell (i.e., at least two of the six seedlings) showed symptoms of wilting.

Field experiments. A preliminary evaluation of grafted watermelon was done in spring 2006 at the CREC, Charleston, SC in a field of Yonges loamy fine sand with pH 6.2. The field was cropped to watermelon the two previous seasons. Symptoms of Fusarium wilt were present in both crops, and *F. oxysporum* f. sp. *niveum* races 1 and 2 were present in the field (35). After disking on 7 March 2006, 10N-10P-10K was broadcast at 897 kg/ha within the rows, and beds were cultivated lightly with a rotovator, shaped 0.9 m wide on 2.7-m centers, and covered with black polyethylene mulch. The herbicide ethalfluralin + clomazone (Strategy 2.1EC; Loveland Products) was applied to the alleys between beds at 1.76 kg/ha on 29 March 2006. The seedless mini-watermelon 'Vanessa' (Sunseeds, now Nunhems USA) was grafted onto 'Emphasis' and 'Macis' bottlegourd and the interspecific hybrid squash 'RS1330' and 'RST04109W', or was not grafted. Transplants were set 0.6 m apart in every other row on 30 March 2006. The herbicide halosulfuron-methyl (Sanda; Gowan) was applied to the alleys at 39 g/ha on 12 May 2006. Cucumber beetles and pickle worms were controlled with esfenvalerate (Asana XL; DuPont Crop Protection) at 34 g/ha on 9 June. Liquid 8N-0P-8K fertilizer was applied daily to the field through the drip irrigation system, beginning 1 week after transplanting (18). The experimental design was a randomized complete block with six replications. Plots were one row wide and 4.6 m long with 3.0 m of nonplanted space between plots within rows. Plants with Fusarium wilt symptoms were counted on 23 May and 5 and 27 June 2006. At the last rating, vines from 12 symptomatic plants, two or more plants per treatment from two different blocks, were cultured on Komada's agar medium as described previously, except that stem pieces were disinfested for 1 min.

In spring 2010 and 2011, the six rootstocks used in the greenhouse experiments were tested in two field experiments after being grafted to seedless watermelon Tri-X 313. The experiments were conducted at the CREC in a field known to be infested with *F. oxysporum* f. sp. *niveum* races 1 and 2 (14,16). In 2010, the naturally occurring inoculum was supplemented with additional inoculum prepared using the race 1 and race 2 isolates used in the greenhouse experiments. These isolates originated from watermelon cropped in the same field in 2005. Inoculum was prepared for each isolate in two clear, autoclavable bags filled with 5 liters of perlite that had been saturated with 2.5 liters of PDB (7). The bags of perlite-broth mixture were autoclaved for 1 h on each of two consecutive days. A microconidial suspension and one 10-cm-diameter plate of PDA with mycelium of the appropriate fungal isolate, cut into pieces, were added to each of the bags, which were incubated at 25°C for 10 days. Inoculum concentration was estimated by dilution plating a suspension of 1 g of perlite in 9 ml of sterile, deionized water onto one-quarter-strength PDA, and by placing 30 pieces of perlite from each bag onto water agar amended with streptomycin at 100 mg/liter. Inoculum concentration of the race 1 and race 2 isolates was 1.0×10^5 and 4.3×10^4 CFU/g, respectively. All 120 pieces of perlite cultured yielded colonies of *F. oxysporum* that resembled the original isolates. Inoculum of the two isolates was combined. In 2010 only, 15 ml (10 g) of inoculum with a mean concentration of 7.3×10^5 CFU/g was added to the transplanting hole immediately before transplanting.

The field was cropped to watermelon in spring 2009 and was seeded to rye in November 2010. The soil type was Yonges loamy fine sand with a pH of 6.4. Prior to bedding, the field was disked twice each spring. On 6 April 2010 and 14 March 2011, raised beds were shaped 0.9 m wide on 2.7-m centers with 15N-0P-15K fertilizer at 336 kg/ha and 10N-10P-10K fertilizer at 448 kg/ha, respectively, banded within the row. Raised beds were formed, sprayed with the herbicides terbacil (Sinbar 80 WP; Tessenderlo Kerley) at 0.22 kg/ha on 7 April 2010 and S-metolachlor (Dual Magnum; Syngenta Crop Protection) at 35 ml/ha on 15 March 2011, then covered with black polyethylene mulch. Liquid 8N-0P-8K fertilizer was applied to the field throughout the season through the drip irrigation system (18). Standard applications of herbicides were made during the season based on weed occurrence, as well as preventative applications of fungicides effective against powdery mildew, gummy stem blight, and downy mildew and preventative applications of insecticides and miticides (17,18).

Triploid watermelon Tri-X 313 was seeded in the greenhouse on 3 March 2010 and 7 March 2011. The same six rootstocks evaluated in the greenhouse were used in the field trial. Rootstocks were seeded in the greenhouse after seedless watermelon had emerged, as described above. Watermelon plants were grafted on 26 March 2010 and 29 March 2011 and transplanted on 15 April 2010 and 13 April 2011. Nongrafted and self-grafted Tri-X 313 were used as susceptible control treatments. Plots were arranged in randomized complete blocks replicated six times. Plots were each 12.1 m long with 12 plants in 2010 and 13.7 m long with 15 plants in 2011, and plants were spaced 0.9 m apart within rows. Plots were separated within rows by 3.0 and 4.6 m of nonplanted space in 2010 and 2011, respectively. The watermelon pollinizer 'SP5' (Syngenta Seeds) was transplanted between every fourth grafted plant to yield three pollinizer plants per plot. Disease incidence was determined by counting the number of plants with symptoms of wilted or dead vines and the number of dead plants on 26 and 29 April; 3, 6, 11, 14, 19, and 26 May; and 2 and 16 June 2010; and 21 and 26 April and 2, 9, 16, 23 and 31 May 2011. On 28 April or 7 May 2010, one symptomatic plant per plot was removed, and two pieces from the stem below the graft union and two pieces from the vine above the first true leaf of the scion were disinfested in 0.5% sodium hypochlorite for 1 min, then cultured on Komada's agar medium. For nongrafted Tri-X 313, pieces of stem and vine were cut from approximately the same sites of the plants as on grafted plants. Marketable fruit were harvested and weighed three times weekly between 23 June and 7 July 2010 and five times weekly between 16 June and 13 July 2011.

Race identification. Fungal isolates recovered from grafted or nongrafted seedless Vanessa watermelon in 2006 were inoculated onto the differential cultivars Black Diamond, Charleston Gray, and Allsweet, which are susceptible to all races of *F. oxysporum* f. sp. *niveum*, resistant to race 0, and resistant to race 1, respectively (45). Inoculum was produced as described previously for the greenhouse inoculation experiments, except that liquid Komada's medium was used (20). The differential cultivars were seeded in a 50:50 sand/perlite medium and grown for 14 days prior to inoculation, when the first true leaf was fully expanded. Seedlings were removed from the growing medium, and the roots were rinsed gently in tap water and dipped into a suspension of 10^5 microconidia/ml. Seedlings were transplanted into Fafard 3B potting mix in 10-cm-diameter pots for each of four pots with four seedlings per pot and held in a greenhouse at approximately 24°C for 21 days. Each seedling was then rated as healthy or diseased, and the percentage of infected seedlings was calculated for each differential cultivar. Pieces of the hypocotyl of one symptomatic Allsweet seedling per pot were cultured on Komada's agar medium to confirm infection by *F. oxysporum*.

Statistical analysis. Data on recovery of *F. oxysporum* from plants in the greenhouse experiments with nongrafted rootstocks were analyzed using SAS PROC MIXED with experiment repetition, inoculation treatment, race, and cultivar as fixed effects, and replication within experiments as a random effect. Percent recovery

ery was transformed with arcsine-square root before analysis. For analysis of percent recovery from both the top and base of each plant, 0 and 100% were replaced with $25/n$ and $100 - 25/n$, respectively, where $n = 20$, the number of plants (38).

Data on recovery of *F. oxysporum* from plants in the greenhouse experiments with grafted rootstocks were analyzed using SAS PROC CATMOD for categorical data, which uses a χ^2 statistic to determine treatment effects. The proportions of plants from which *F. oxysporum* was or was not recovered (a binary response) were transformed by calculating the logit, and the linear model parameters were then estimated using the maximum likelihood option. In order to get models to converge (i.e., arrive at a unique solution) that included zero values for some categories, observed proportions were replaced with a Bayes estimator calculated as $x = (ct + k/r) \times n/(n + k)$, where x = the transformed proportion, ct = count of plants with (or without) *F. oxysporum*, $r = 2$ (for a binary response), k = a constant (which was set equal to r), and n = total number of plants per treatment combination (37). Stepwise model reduction was done to improve the sensitivity of the analysis. When treatment effects were significant, contrast statements were written to compare treatment levels. Two-way interactions of inoculation and cultivar were examined by including nested-by-value effects in model statements to compare inoculation treatments within cultivars. Proportions were generated using the predict option to back-transform logit values. Categorical analysis of variance also was used to examine the proportions of pathogenic and nonpathogenic isolates recovered from plants inoculated in the greenhouse, and the proportions of plants from which *F. oxysporum* was recovered in the 2006 field experiment.

Disease and yield data from field experiments were analyzed with SAS PROC MIXED, with treatment as a fixed effect and block as a random effect. Before analysis, disease incidence percentages for the early season in 2010 and the early and late season in 2011 were transformed with arcsine of the square root to correct

for non-normality and inequality of variances among treatments. Back-transformed means are shown in the tables. When yield data from the two experiments were combined, year, treatment, and the year-treatment interaction were tested as fixed effects and block-year as a random effect. Preplanned, single-degree-of-freedom contrasts were used to compare rootstock genera and to compare rootstocks with watermelon treatments.

Results

Disease development in greenhouse experiments. In the four experiments with nongrafted plants, all noninoculated control plants were symptomless (i.e., no plants wilted) and all inoculated bottlegourd and interspecific hybrid squash plants also were symptomless. Tri-X 313 had 65 to 100% symptomatic plants across the four experiments. Allsweet (resistant to race 1) was symptomless when inoculated with race 1 and had 70% symptomatic plants when inoculated with race 2 in experiment 1. This cultivar was uncharacteristically 100% symptomless in experiment 2 when inoculated with race 2. Ojakkyo citron plants showed typical symptoms of wilting when inoculated with race 1 or race 2 in the first experiment (100% of inoculated plants for each race), although there were no symptoms in the second experiment with race 2. *F. oxysporum* was recovered from the base of all symptomatic plants. The fungus was recovered from 100% of the tops of Tri-X 313 plants and from 86 to 100% of the tops of symptomatic Ojakkyo plants.

In the four experiments with grafted plants, all noninoculated control plants were symptomless. The nongrafted Tri-X 313 control had 40 to 80% symptomatic plants when inoculated with race 1 and $\geq 90\%$ symptomatic plants when inoculated with race 2. Nongrafted Allsweet watermelon was symptomless when inoculated with race 1 and had 15 to 60% symptomatic plants when inoculated with race 2. Self-grafted Tri-X 313 plants had 55 and 75% wilt incidence when inoculated with race 1 and race 2, respectively. Tri-X 313 scions grafted onto Ojakkyo plants showed typical symptoms of wilting when inoculated with race 1 (60% of the plants) and race 2 (20 to 30% of the plants) (Fig. 1). *F. oxysporum* was recovered from 100% of the rootstock portions (bases) of symptomatic plants. The species also was recovered from 100% of the scions on all symptomatic plants grafted onto Ojakkyo and from nongrafted Tri-X 313 and Allsweet watermelon. However, recovery from the scions of symptomatic, self-grafted Tri-X 313 was 73% for race 1 inoculations and 80% for race 2 inoculations. In addition, one scion grafted onto 'Strong Tosa' showed wilt symptoms when inoculated with race 1 in experiment 1, and two scions grafted onto Macis showed wilt symptoms when inoculated with race 2 in experiment 2. *F. oxysporum* was isolated from the Strong Tosa rootstock and from one of the two Macis rootstocks but was not isolated from the three scions.

Recovery of *F. oxysporum* from noninoculated plants. In addition to recovery from symptomatic plants, *F. oxysporum* was recovered from the bases of 0 to 40% of the noninoculated control plants in six of the eight experiments. In the experiments with nongrafted plants inoculated with race 1, percent recovery of *F. oxysporum* was not significantly >0 based on t tests ($P \geq 0.25$; Fig. 2A). In the experiments with nongrafted plants inoculated with race 2, there was an inoculation-experiment interaction ($P = 0.002$). Percent recovery from noninoculated plants of Macis, 'WMXP 3945', 'Shintosa Camel', Strong Tosa, and Tri-X 313 was significantly >0 in the first experiment (Fig. 2B) and percent recovery from Ojakkyo was significantly >0 in the second experiment (t tests, $P < 0.05$; Fig. 2C).

With grafted plants inoculated with race 1, percent recovery of *F. oxysporum* from bases of the rootstocks or the nongrafted watermelon control plants was not significantly >0 for any cultivar in experiment 1 (single-degree-of-freedom contrasts, $P \geq 0.34$; Fig. 3A). In experiment 1 with race 2 and grafted plants, percent recovery was significantly >0 for Emphasis and WMXP 3945 bottlegourds and for Ojakkyo citron but not for the other cultivars ($P \leq 0.04$; Fig. 3C). In experiment 2 for each race, percent recovery of

Fig. 1. Wilted Tri-X 313 seedless watermelon scion on wilted Ojakkyo citron rootstock that had been inoculated with *Fusarium oxysporum* f. sp. *niveum* in a greenhouse after grafting.

F. oxysporum from the bases of rootstocks was not significantly >0 for any cultivars (Fig. 3B and D).

F. oxysporum was recovered from the tops of 2 of 640 (0.3%) noninoculated, nongrafted plants and 0 to 3 (mean of 2.4%) inoculated, nongrafted plants when the fungus was not recovered from the bottom of the same plant. There was no significant difference among cultivars for recovery from only the top of inoculated plants ($P = 0.81$). Similarly, in the experiments with grafted plants, *F. oxysporum* was recovered from 0 to 2 scions of noninoculated or inoculated plants of each cultivar summed across the four experiments. Based on categorical ANOVA, percent recovery from scions of plants did not differ between inoculated and noninoculated grafted plants or among cultivars ($\chi^2 = 0.0$, $P = 1.00$).

Of the 73 isolates recovered from the bases or tops of noninoculated plants, 72 were nonpathogenic when tested on the Fusarium wilt-susceptible Sugar Baby (Table 2). The single pathogenic isolate was recovered from the base of a symptomless, grafted Ojakkyo plant in the first experiment with race 2. In all pathogenicity tests, the race 1 and race 2 isolates used in this study and included as positive control treatments were pathogenic, whereas the noninoculated Sugar Baby seedlings remained symptomless.

Recovery of *F. oxysporum* from bases of inoculated plants in greenhouse experiments. There was a race–cultivar interaction ($P < 0.0001$) for the percent recovery of *F. oxysporum* from the bases of inoculated, nongrafted plants. However, for both races, percent recovery from inoculated plants was greater than from noninoculated plants for each cultivar ($P < 0.02$ for race 1 and $P \leq 0.001$ for race 2; Fig. 2). Thus, *F. oxysporum* f. sp. *niveum* races 1 and 2 colonized the bases of inoculated, nongrafted plants. Percent recovery from bases of Tri-X 313, Ojakkyo, and Allsweet plants inoculated with race 1 was greater than from all bottlegourd and interspecific hybrid squash cultivars, which did not differ from each other ($P < 0.01$; Fig. 2A). When nongrafted plants were inoculated with race 2, percent recovery of *F. oxysporum* was 79 to 100% (Fig. 2B and C). Frequency of recovery did not differ among cultivars ($P = 0.69$ and $P = 0.29$ for cultivar effects in experiments 1 and 2, respectively).

When grafted plants were inoculated with *F. oxysporum* f. sp. *niveum*, there was a race–experiment interaction ($P < 0.02$). In both experiments with race 1 and in experiment 2 with race 2, percent recovery of *F. oxysporum* was greater from inoculated Ojakkyo as well as nongrafted and self-grafted Tri-X 313 plants than from the corresponding noninoculated cultivars ($P < 0.01$), whereas percent recovery did not differ between inoculated and noninoculated plants of bottlegourd and interspecific hybrid squash (Fig. 3A, B, and D). In experiment 1 with race 2, percent recovery differed between inoculated and noninoculated treatments for nongrafted Tri-X 313 and Strong Tosa ($P \leq 0.03$) but not for the other cultivars (Fig. 3C).

With both races, *F. oxysporum* was isolated more frequently from the bases of inoculated, nongrafted (control) plants of Tri-X 313 and grafted Ojakkyo than from inoculated, grafted bottlegourd and interspecific hybrid squash rootstocks ($P \leq 0.05$) (Fig. 3). The only exception was with race 2 in experiment 1, when frequency of recovery from Shintosa Camel plants was not significantly different from that of Tri-X 313 ($P = 0.09$; Fig. 3C). In the two experiments that included self-grafted Tri-X 313 as a grafting control treatment, recovery was as great from self-grafted plants as from nongrafted plants of Tri-X 313 (Fig. 3B and D).

Recovery of *F. oxysporum* from the top and base of inoculated plants. As reported above, *F. oxysporum* was recovered infrequently from the tops of inoculated plants from which the fungus was not recovered from the base of the same plant. Thus, the percentage of plants from which *F. oxysporum* was recovered from both the base and the top of the same plant was considered the most appropriate measure of colonization. *F. oxysporum* was not recovered from both the top and bottom of any noninoculated, nongrafted plants in any of the four experiments. With race 1 inoculation, percent recovery was greater from inoculated plants than from noninoculated plants of Allsweet, Macis, Shintosa Camel,

Ojakkyo, and Tri-X 313 ($P \leq 0.01$). With race 2 inoculation, percent recovery was greater from inoculated plants than noninoculated plants of Allsweet, Ojakkyo, Strong Tosa, and Tri-X 313 in the first experiment, and for all cultivars except Allsweet in the second experiment.

The frequency of recovery of *F. oxysporum* from the top and base of nongrafted plants inoculated with each race was greater for

Fig. 2. Percent recovery of *Fusarium oxysporum* from the base of nongrafted plants that were not inoculated or were inoculated with *F. oxysporum* f. sp. *niveum* in a greenhouse. Each mean is based on 20 plants arranged as four plants in each of five replicate pots in each of two experiments. Cultivar abbreviations are shown in Table 1. Percent recovery from inoculated plants was greater than from noninoculated plants for each cultivar ($P < 0.02$). **A**, Inoculation with race 1 of *F. oxysporum* f. sp. *niveum*, for which there was no significant difference between the two experiments. Means for inoculated cultivars with the same letter were not significantly different based on t tests ($P < 0.0001$). Means for noninoculated cultivars did not differ significantly. With race 2 of the pathogen, results of **B**, experiment 1 and **C**, experiment 2 differed because there was an inoculation–experiment interaction ($P = 0.002$), with greater recovery of *F. oxysporum* from noninoculated plants in experiment 1 than in experiment 2. In both experiments with race 2 (**B** and **C**), percent recovery of *F. oxysporum* from plants did not differ among cultivars within inoculation treatments. Refer to the main text for details of how *F. oxysporum* was recovered from plants.

the susceptible watermelon control treatment than for the three bottlegourd and two interspecific hybrid squash rootstocks ($P \leq 0.01$; Fig. 4). Note that the susceptible watermelon control was Tri-X 313 in the race 1 experiments and the second race 2 experiment, whereas Black Diamond served this purpose in the first experiment with race 2. There was a significant interaction among race, cultivar, and experiment, primarily due to different responses of Ojakkyo and Allsweet. With race 1 inoculation, frequency of recovery of *F. oxysporum* from Ojakkyo was less than from Tri-X 313 ($P = 0.01$; Fig. 4A). With race 2 inoculation, recovery of *F. oxysporum* from Ojakkyo did not differ from that of Black Diamond in experiment 1 ($P = 0.055$) but was less than recovery from Tri-X 313 in experiment 2 ($P < 0.0001$) (Fig. 4B). In both race 1 experiments and in the first experiment with race 2, recovery from Ojakkyo was greater than recovery from the other five rootstock cultivars and from Allsweet (Fig. 4). In the second experiment with race 2, there were no differences in recovery of *F. oxysporum* between Ojakkyo and the other cultivars (Fig. 4B). With race 1 inoculation, the frequency of recovery from Allsweet, which is resistant to race 1, did not differ from recovery from the bottlegourd and interspecific hybrid squash cultivars (Fig. 4A). However, recovery of *F. ox-*

ysporum from Allsweet differed between the two experiments with the race 2 isolate of *F. oxysporum* f. sp. *niveum* (Fig. 4B). In the first experiment, recovery from Allsweet was greater than recovery from the bottlegourd and interspecific hybrid squash cultivars, except Strong Tosa ($P \leq 0.006$). In the second experiment with race 2, percent recovery of *F. oxysporum* from Allsweet did not differ from that of any rootstock cultivars.

Percent recovery of *F. oxysporum* from both the scion and rootstock portions of grafted plants did not differ between race 1 and race 2 isolates of the pathogen ($P = 0.23$). However, there was a significant inoculation-cultivar interaction ($P < 0.0001$). *F. oxysporum* was recovered more frequently from inoculated Allsweet, Ojakkyo, self-grafted Tri-X 313, and nongrafted Tri-X 313 than from the corresponding noninoculated cultivars ($P \leq 0.0003$; Fig. 5). Percent recovery was low for bottlegourd and interspecific hybrid squash rootstocks, and inoculated and noninoculated plants did not differ significantly for these five cultivars in terms of recovery of *F. oxysporum* ($P \geq 0.49$).

A separate analysis was done with data only from the inoculated, grafted plants because of the inoculation-cultivar interaction. Frequency of recovery was consistent between experiments and

Fig. 3. Percent recovery of *Fusarium oxysporum* from the base of grafted plants that were not inoculated or were inoculated with *F. oxysporum* f. sp. *niveum* in a greenhouse. Missing white bars indicate no recovery. Cultivar abbreviations are shown in Table 1; TG = Tri-X 313 self-grafted control treatment. Percent recovery did not differ among noninoculated cultivars without letters shown. **A**, In experiment 1 with race 1 of *F. oxysporum* f. sp. *niveum*, percent recovery from inoculated cultivars with the same letter did not differ significantly based on *t* tests ($P \leq 0.008$). **B**, In experiment 2 with race 1, percent recovery from inoculated cultivars with the same letter did not differ significantly based on *t* tests ($P \leq 0.005$). **C**, In experiment 1 with race 2, percent recovery from inoculated cultivars with the same lowercase letter did not differ significantly based on *t* tests ($P \leq 0.05$). Percent recovery from noninoculated cultivars with the same uppercase letter did not differ significantly based on *t* tests ($P \leq 0.05$). **D**, In experiment 2 with race 2, percent recovery of *F. oxysporum* from inoculated cultivars with the same letter did not differ significantly based on *t* tests ($P \leq 0.002$). Refer to the main text for details on the isolation method used to recover *F. oxysporum*.

between races, because there were no significant main effects or interactions between experiment and race main effects ($P > 0.18$). Percent recovery from scions (or tops) and rootstocks (or bases of plants) of nongrafted Tri-X 313, self-grafted Tri-X 313, nongrafted Allsweet, and grafted Ojakkyo was significantly greater than from the five bottlegourd and interspecific hybrid squash rootstocks ($P \leq 0.01$; Fig. 5). Percent recovery from Tri-X 313 and Ojakkyo did not differ, and recovery from both was greater than recovery from Allsweet ($P \leq 0.01$). Percent recovery from self-grafted Tri-X 313 was greater than from bottlegourd and interspecific hybrid squash rootstocks but less than from nongrafted Tri-X 313 ($P \leq 0.01$).

In all, 6 of 7 isolates recovered from the tops of nongrafted, inoculated plants and 9 of 14 isolates recovered from the scions of grafted, inoculated plants were pathogenic on watermelon (i.e., were identified as *F. oxysporum* f. sp. *niveum*); 2 of 9 isolates recovered from the rootstocks of grafted, inoculated plants were pathogenic (Table 2). All four isolates recovered from the stem above the cotyledons on inoculated, nongrafted Tri-X 313 and Allsweet plants were pathogenic. In a categorical ANOVA, the proportion of pathogenic isolates among all recovered isolates was greater from inoculated than noninoculated plants ($P = 0.001$) and greater from the tops than the bases of plants ($P = 0.02$). The predicted probability of recovering a pathogenic isolate from the base of noninoculated plants was 0.03 ± 0.02 (mean $\pm$ standard error), whereas the predicted probability of recovering a pathogenic isolate from inoculated plants was 0.28 ± 0.13 from the base and 0.69 ± 0.10 from the top.

Field experiments. In the 2010 and 2011 field experiments, symptoms of *Fusarium* wilt appeared by 8 days after transplanting. Disease progression differed between years; therefore, data from each year were analyzed separately. In 2010, when inoculum was added to the transplanting holes, *F. oxysporum* f. sp. *niveum* readily infected the roots of young plants. Disease progressed rapidly for the first 3 weeks after transplanting and then reached a plateau for the rest of the season (Fig. 6). Self-grafted Tri-X 313 had the greatest incidence of *Fusarium* wilt for the entire 2010 growing season, and plants grafted onto Ojakkyo citron had the second greatest incidence (Fig. 6). In 2011, plants grafted onto Ojakkyo had the greatest disease incidence 19 days after transplanting but nongrafted Tri-X 313 had the greatest incidence for the rest of the growing season (Table 3; Fig. 6). In both years, plants grafted onto Ojakkyo showed symptoms within 4 weeks after transplanting, and wilt incidence increased $<5\%$ after that time (Fig. 6).

At 48 days after transplanting, $>50\%$ of the self-grafted Tri-X 313 plants and plants grafted onto Ojakkyo showed wilt symptoms in 2010, compared with 45.8% wilt incidence for Tri-X 313 nongrafted plants; whereas, in 2011, only 21.9% of Ojakkyo

grafted plants had wilt symptoms and 25.6% of self-grafted Tri-X 313 plants were wilting, compared with $>50\%$ of the nongrafted Tri-X 313 plants which were wilted (Fig. 6). In both years, more Tri-X 313 plants (averaged across both control treatments) showed

Fig. 4. Percent recovery of *Fusarium oxysporum* from both the base and the top of nongrafted plants 3 weeks after inoculation with race 1 or race 2 of *F. oxysporum* f. sp. *niveum* in four greenhouse experiments. Cultivar abbreviations are shown in Table 1. Each mean is based on 20 plants arranged as four plants in each of five replicate pots. Values are back-transformed from arcsine-square roots, where a value of 1.25 is equivalent to 0 of 20 plants. **A**, For race 1 inoculations across the two experiments, cultivars with the same letter are not significantly different based on *t* tests ($P \leq 0.001$). **B**, For race 2, Black Diamond was substituted for Tri-X 313 in experiment 1. Cultivars with the same letter are not significantly different based on *t* tests ($P \leq 0.006$ for experiment 1, designated with lowercase letters, and $P < 0.0001$ for experiment 2, designated with uppercase letters). Refer to the main text for details of how *F. oxysporum* was recovered from plants.

Table 2. Pathogenicity to Sugar Baby watermelon of isolates of *Fusarium oxysporum* that were recovered from noninoculated plants or plants inoculated with *F. oxysporum* f. sp. *niveum* in eight greenhouse experiments^x

Grafting	Race	Experiment	Inoculation	Recovered from top or scion of plant ^y		Recovered from base of plant ^z	
				Number of isolates	Number pathogenic	Number of isolates	Number pathogenic
Nongrafted	1	1	Control	n.d.	n.d.	11	0
Nongrafted	2	1	Control	1	0	23	0
Nongrafted	2	2	Control	n.d.	n.d.	3	0
Grafted	1	1	Control	1	0	8	0
Grafted	2	1	Control	3	0	22	1
Grafted	2	2	Control	1	0	n.d.	n.d.
Nongrafted	1	2	Inoculated	4	3	n.d.	n.d.
Nongrafted	2	2	Inoculated	3	3	n.d.	n.d.
Grafted	1	1	Inoculated	1	0	3	2
Grafted	1	2	Inoculated	6	6	n.d.	n.d.
Grafted	2	1	Inoculated	5	2	6	0
Grafted	2	2	Inoculated	2	1	n.d.	n.d.

^x Pathogenic isolates caused wilting or death of four or more of six watermelon seedlings 14 days after seeds were inoculated with a microconidial suspension of the *F. oxysporum* isolate when sown into potting soil; n.d. = not done.

^y Isolates were obtained from surface-disinfested pieces of the stem between the first and second true leaf of nongrafted plants or the scion of grafted plants, as detailed in the main text.

^z Isolates were obtained from surface-disinfested pieces cut approximately 1 cm above the soil line from the stem of nongrafted plants or the rootstock portion of grafted plants, as detailed in the main text.

symptoms than scions grafted onto bottlegourd or interspecific hybrid squash rootstocks (single-degree-of-freedom contrasts, $P < 0.05$ and $P < 0.0001$ in 2010 and 2011, respectively). Disease incidence did not differ between self-grafted Tri-X 313 and Ojakkyo (Table 3). In both years, there were no differences between bottlegourd and interspecific hybrid squash rootstocks and no differences among cultivars within each rootstock genus. In 2010, self-grafted Tri-X 313 plants had a greater late-season disease incidence than all other treatments except Ojakkyo citron ($P \leq 0.05$;

Table 3). In addition, more grafted Ojakkyo citron plants showed symptoms of wilt than grafted Emphasis plants. In 2011, nongrafted Tri-X 313 had more wilted plants late in the season than any other treatment except self-grafted Tri-X 313.

For weight, number, and average size of fruit, there were significant effects of year ($P < 0.0001$ for each measurement) and treatment ($P < 0.0001$, $P = 0.0070$, and $P = 0.0131$, respectively) but no significant interactions ($P > 0.81$); therefore, yield data were combined. Yields were almost twice as great in 2011 as in 2010. Mean weight of marketable fruit harvested was 29.8 and 56.8 Mg/ha in 2010 and 2011, respectively, and mean number of fruit per hectare was 3,984 and 8,183 in 2010 and 2011, respectively. In general, grafting watermelon onto cucurbit rootstocks increased weight and number of fruit (single-degree-of-freedom contrasts between watermelon control treatments and cucurbit rootstocks: $P = 0.0002$ for weight and $P = 0.007$ for number of fruit). All three grafted bottlegourd (*Lagenaria* spp.) cultivars produced a greater marketable fruit weight than nongrafted and self-grafted Tri-X 313 (Table 4). Tri-X 313 grafted onto interspecific hybrid squash Strong Tosa also yielded more fruit by weight than the Tri-X 313 control treatments. Weight of fruit produced by grafted Ojakkyo plants did not differ significantly from that of nongrafted and self-grafted Tri-X 313 plants. Watermelon grafted onto Emphasis produced a greater number of fruit than watermelon grafted onto Shintosa Camel, Ojakkyo, and the watermelon control treatments (Table 4). When mean fruit numbers across rootstock genera were compared with single-degree-of-freedom contrasts, watermelon grafted onto bottlegourd rootstocks produced more fruit per hectare ($n = 6,949$) than watermelon grafted onto Ojakkyo citron ($n = 5,555$, $P = 0.014$) or interspecific hybrid squash rootstocks ($n = 5,865$, $P = 0.015$).

Watermelon grafted onto bottlegourd, interspecific hybrid squash, and citron rootstocks produced larger fruit than the two watermelon control treatments (single-degree-of-freedom contrasts, $P \leq 0.01$). Watermelon grafted onto citron also yielded larger fruit than watermelon grafted onto bottlegourd (Table 4). The size of fruit produced on interspecific hybrid squash rootstocks was intermediate to the size of fruit produced on citron and bottlegourd rootstocks. Watermelon grafted onto bottlegourd and interspecific hybrid squash rootstocks produced fruit between 0.3 and 0.7 kg larger than the ideal watermelon fruit size of 6.8 kg, and fruit produced on the citron rootstock was 1.0 kg larger (34).

Recovery of *F. oxysporum* and *F. oxysporum* f. sp. *niveum* from field-grown, grafted plants. In all, 12 isolates of *F. ox-*

Fig. 5. Percent recovery of *Fusarium oxysporum* from both the base of the rootstock portion and the Tri-X 313 scion on grafted plants 3 weeks after inoculation with race 1 or race 2 of *F. oxysporum* f. sp. *niveum* in a greenhouse. Cultivar abbreviations are shown in Table 1; TG = Tri-X 313 self-grafted control treatment. Cultivars were grafted with seedless watermelon Tri-X 313, except the nongrafted Tri-X 313 (TX) and nongrafted Allsweet (AS) control treatments. Each value is a mean across four experiments, two each with race 1 and race 2 isolates, because there were no significant interactions among cultivar, race, or experiment effects in the analysis. Percent recovery from inoculated cultivars with the same letter did not differ significantly based on χ^2 tests ($P \leq 0.01$). Percent recovery did not differ among noninoculated cultivars. Refer to the main text for details of how *F. oxysporum* was recovered from plants.

Fig. 6. Progression of *Fusarium* wilt symptoms on seedless Tri-X 313 watermelon grafted onto each of six rootstocks, self-grafted, or not grafted and planted in a field in Charleston, SC infested with a mixture of races 1 and 2 of *Fusarium oxysporum* f. sp. *niveum* in spring 2010 and 2011. The legend shows rootstock cultivar names. Refer to Table 1 for details of each cultivar. Each data point is the mean and standard error of six observations.

oxysporum recovered in 2006 from grafted plants (10 isolates) or from nongrafted control plants (2 isolates) were tested for pathogenicity on three differential cultivars to determine whether the isolates were *F. oxysporum* f. sp. *niveum* and, if so, which races. In total, 5 of the 10 isolates from scions of grafted plants and 1 of the 2 isolates from vines of nongrafted plants were *F. oxysporum* isolates that were not pathogenic on watermelon, because they caused no symptoms on any of the cultivars. The remaining five isolates from grafted plants were race 1 and the remaining isolate from the nongrafted plant was race 2. The known isolate of race 2 included as a control isolate caused $\geq 75\%$ wilt incidence on all three cultivars. *F. oxysporum* was recovered from all symptomatic Allsweet seedlings.

In 2010, *F. oxysporum* was recovered from 17 to 100% of the symptomatic plants sampled in the field (Table 5). Recovery from nongrafted Tri-X 313 was 100% from both the top and base of the plants. Recovery did not differ significantly among cultivars (χ^2 , $P = 0.31$) or between the top and base of plants (χ^2 , $P = 0.07$). Across the eight grafting treatments, 23 and 34 of the 35 plants that were infected yielded *F. oxysporum* from the top or scion and from the base, respectively. Only one plant, a grafted Strong Tosa plant, yielded *F. oxysporum* only from the scion and not from the base of the rootstock. The forma specialis and race of *F. oxysporum* isolates collected in 2010 were not determined.

Discussion

The susceptible watermelon and citron cultivars used in this study showed typical symptoms of wilting when inoculated with two races of *F. oxysporum* f. sp. *niveum* in a greenhouse. *F. oxysporum* was reisolated from 87 to 100% of the top portions and from 100% of the bases of the wilted, nongrafted, or grafted susceptible cultivars. However, the resistant bottlegourd and interspecific hybrid squash cultivars did not display symptoms even when the plants were infected by *F. oxysporum* f. sp. *niveum* in some experiments or replications. The five cultivars of bottlegourd and interspecific hybrid squash evaluated fit the definition of “asymptomatic hosts” recently proposed by Malcolm et al. (28), because they were infected by *F. oxysporum* f. sp. *niveum* but did not show disease symptoms. Asymptomatic infection of various plant hosts is common with *F. oxysporum* (23). Thus, infection cannot be used as the sole criterion for evaluating resistance of rootstock cultivars.

F. oxysporum infects host roots through unsubsized root tips (6). The distribution of *F. oxysporum* in planta was illustrated recently by use of colorimetric reagents that reacted with enzymes produced by the pathogen (5). When *F. oxysporum* f. sp. *conglutinans* infected roots of susceptible mutants of *Arabidopsis*, the fungus rapidly progressed to the vascular cylinder. Resistance to wilt was expressed as reduced root tip colonization or as a restriction of

pathogen growth into the vascular cylinder (5). Because *F. oxysporum* f. sp. *niveum* was recovered from the stem bases on rootstock cultivars in the current study, the fungus clearly had progressed into the plant vascular system beyond the roots. Resistance of bottlegourd and interspecific hybrid squash to *F. oxysporum* f. sp. *niveum* may involve mechanisms other than restriction of vascular tissue colonization, such as formation of tyloses that block or restrict movement of *F. oxysporum* after the fungus has entered the vascular cylinder (6,24). Alternatively, it is possible that infection of the rootstock could trigger a resistant reaction in the scion (10). For example, Kuniyasu and Takeuchi (24) observed tyloses that had formed in a watermelon scion in response to infection of a bottlegourd rootstock by *F. oxysporum* f. sp. *lagenariae*.

Cucurbit rootstocks and watermelon can be infected by nonpathogenic *F. oxysporum*. Both noninoculated and inoculated nongrafted and grafted rootstock and watermelon plants were colonized by nonpathogenic *F. oxysporum* in this study. Similarly, 49% of 153 isolates of *F. oxysporum* recovered from surface-disinfested watermelon plants with symptoms of Fusarium wilt in Maryland and Delaware and 74% of isolates recovered from surface-disinfested watermelon seed in Tunisia were nonpathogenic on watermelon (9,45). Because *F. oxysporum* was recovered from one sample of the brand of potting soil used in the greenhouse experiments in this study (A. P. Keinath, unpublished data), the potting soil likely was a source of the nonpathogenic *F. oxysporum* recovered from greenhouse plants. Recovery of *F. oxysporum* from all inoculated watermelon plants and from most inoculated rootstocks was greater than from noninoculated plants, which means that colonization of plants by *F. oxysporum* f. sp. *niveum* was greater than by other *F. oxysporum* isolates present in the potting soil. Roots were exposed to 10^6 conidia/ml when dipped in the conidial suspension of *F. oxysporum* f. sp. *niveum* used to inoculate plants before the roots were exposed to *F. oxysporum* at 30 CFU/g, present naturally in the potting soil (data not shown). Thus, even though isolates of *F. oxysporum* not pathogenic on watermelon have been shown to induce systemic resistance to Fusarium wilt in watermelon, the much greater inoculum density and prior exposure to the pathogen in the experiments in this study likely overcame any beneficial effects of root infection by other *F. oxysporum* isolates from the potting medium (25). It is possible that some of the nonpathogenic isolates of *F. oxysporum* recovered from noninoculated plants in the greenhouse experiments would not have been isolated if a longer root disinfestation treatment had been used. *F. oxysporum* f. sp. *niveum* is relatively insensitive to sodium hypochlorite; inside watermelon stems, the fungus can survive up to 5 min in a 0.5% sodium hypochlorite solution (21).

With a few minor exceptions, colonization of plants inoculated with *F. oxysporum* f. sp. *niveum* in the greenhouse experiments fit

Table 3. Incidence of Fusarium wilt on seedless watermelon Tri-X 313 that was grafted onto six cucurbit rootstocks, self-grafted, or not grafted and planted into a field infested with *Fusarium oxysporum* f. sp. *niveum* races 1 and 2 in 2010 and 2011 in Charleston, SC

Rootstock cultivar and treatment ^z	Wilt incidence (%) ^w			
	Early season ^x		Late season ^y	
	2010	2011	2010	2011
Tri-X 313 self-grafted	62.4 a	9.3 ab	68.6 a	25.6 ab
Tri-X 313, nongrafted	36.9 ab	8.0 ab	45.8 bc	52.9 a
Ojakkyo grafted	51.5 ab	19.7 a	55.6 ab	21.9 b
Strong Tosa grafted	34.0 b	0.9 bc	44.4 bc	0.8 c
Shintosa Camel grafted	31.3 b	0.2 c	41.7 bc	0.2 c
WMXP 3945 grafted	26.5 b	1.1 bc	37.5 bc	1.9 c
Macis grafted	46.7 ab	0.2 c	48.6 bc	1.7 c
Emphasis grafted	29.2 b	0.0 c	31.9 c	0.2 c
P value	0.0059	0.0001	0.0250	0.0001

^w Means are least-squares means back-transformed from arcsine of the square root values used in the analysis of variance, except for 2010 late-season incidence data, which did not require transformation. Means within a column followed by the same letter are not significantly different based on *t* tests at $P \leq 0.01$, except for final wilt incidence in 2010, where $P \leq 0.05$.

^x Early season ratings were taken 18 days after transplanting in 2010 and 19 days after transplanting in 2011.

^y Late season ratings were taken 48 days after transplanting in each of 2010 and 2011.

^z Refer to Table 1 for details of each cucurbit cultivar.

two distinct patterns. In the first pattern, susceptible cultivars of watermelon and Ojakkyo citron yielded *F. oxysporum* frequently from bases and tops or scions, whereas recovery from bottlegourd and interspecific hybrid squash was infrequent or less frequent. This pattern was observed in all four experiments with grafted plants and in the two experiments with nongrafted rootstock cultivars inoculated with race 1 of *F. oxysporum* f. sp. *niveum*. The second pattern was uniform infection of the bases of nongrafted plants of both susceptible and resistant cultivars by race 2 but with less frequent infection of the tops of resistant cultivars. Thus, the race 2 isolate used in these experiments was capable of infecting the bases of rootstocks more frequently than the race 1 isolate used. Race 2 isolates have been reported to be more virulent on watermelon than race 1 isolates (29,45). It appears that this virulence includes a greater degree of colonization of asymptomatic hosts (28).

To determine whether grafted plants have been infected by *F. oxysporum* f. sp. *niveum*, sampling from the scion is more likely to yield pathogenic isolates than sampling from the rootstock, based on results of this study. Based on the greenhouse results, 69% of the isolates recovered from the tops or scions of inoculated plants were pathogenic, while only 28% of the isolates recovered from the bases of inoculated plants were pathogenic. In a smaller sample of isolates that were collected from the field in 2006, 50% of the isolates from scions or tops were pathogenic.

The different results in the 2010 and 2011 field experiments, in which initial inoculum levels differed greatly, paralleled results reported by Martyn and McLaughlin (31) for nongrafted watermelon and by Caperton et al. (2) for yellow summer squash. When both crops were inoculated with race 1 of *F. oxysporum* f. sp. *niveum*, cultivars appeared more susceptible as the inoculum density increased. The frequency of infection of scions was greater in the field in 2010 than in the greenhouse experiments, where infection of scions grafted onto bottlegourd and interspecific hybrid squash rootstocks was infrequent. It is likely that continuous exposure of roots to inoculum in the root zone of the transplants in the field increased the frequency of infection compared with a brief dipping of roots into a suspension of microconidia of the pathogen in the greenhouse. Infection of bottlegourd and interspecific hybrid squash was similar to the observations reported by Martyn and McLaughlin (32) and summarized by Martyn (30), in that some isolates of *F. oxysporum* f. sp. *niveum* could infect melon, cucumber, and summer squashes (*C. pepo*), and young host plants (other than watermelon) were more susceptible than older plants. Zhou and Everts (48) found that 4 of 88 isolates of *F. oxysporum* f. sp. *niveum* were pathogenic on muskmelon (*Cucumis melo*) seedlings. One of these isolates caused a moderate level of wilt (20 to 35% incidence) on mature muskmelon plants in the field.

Despite colonization of the plants by *F. oxysporum* f. sp. *niveum* in some experiments, the five cultivars of bottlegourd and interspecific hybrid squash tested in this study can be considered resistant

to this pathogen (i.e., did not show symptoms of wilting) and, in the greenhouse, had little to no recovery of *F. oxysporum* from the tops of plants. A limited incidence of recovery of *F. oxysporum* f. sp. *niveum* from the upper portion of stems of resistant watermelon cultivars has been reported (46). *Cucurbita moschata* and *C. maxima*, the parents of interspecific hybrid squash rootstocks, previously were shown to be resistant to *F. oxysporum* f. sp. *niveum* (33). Similarly, in a greenhouse study in Turkey, Macis, Emphasis, and Strong Tosa were listed as resistant to *F. oxysporum* f. sp. *niveum* races 0, 1, and 2 (44). In an infested field, watermelon grafted onto interspecific hybrid squash rootstocks, including Strong Tosa, and bottlegourd rootstocks, including Emphasis, showed no symptoms, whereas 5 to 10% of the nongrafted watermelon plants did develop symptoms (44). In contrast, Macis and Strong Tosa (referred to as Strong Tosa in that study) evaluated as rootstocks and grafted plants were susceptible to three isolates of *F. oxysporum* f. sp. *niveum* from Tunisia, whereas Emphasis and Shintosa Camel (referred to as S. Camel) were resistant to very resistant (1). Macis and Emphasis were listed as cultivars of interspecific hybrid squash, which was assumed to be an error in that report (1).

Watermelon grafted onto Ojakkyo had moderate to severe Fusarium wilt incidence in the field and greenhouse experiments in this study and frequently was colonized by *F. oxysporum* f. sp. *niveum* in the greenhouse trials. Because citron has greater resistance to *Meloidogyne incognita* than *L. siceraria*, hybrid *Cucurbita*, or watermelon, citron rootstocks could be useful in fields infested with southern root-knot nematodes (39). However, before being used in fields infested with *F. oxysporum* f. sp. *niveum*, citron rootstock cultivars must be screened for resistance to *F. oxysporum* f. sp. *niveum*. Based on a recent report of a high level of resistance to Fusarium wilt caused by race 2 in citron United States Department of Agriculture Plant Introduction genotypes, it should be possible to find resistant lines that could be used as rootstocks (42).

With watermelon, an increase in yield based on marketable fruit weight results from an increase in the number of marketable fruit, the size of marketable fruit, or both (13). The increase in marketable fruit weight produced by watermelon grafted onto bottlegourd observed in this study mainly was due to an increase in the number of fruit, not an increase in the size of fruit. The same result was obtained in field trials in Turkey with a diploid watermelon cultivar grafted onto the bottlegourd rootstock 'Skopje' and planted into soil with a low inoculum level of *F. oxysporum* f. sp. *niveum* (43,44). In addition, grafting onto Emphasis increased the number

Table 5. Recovery of *Fusarium oxysporum* from the base of the rootstock and the Tri-X 313 scion of grafted watermelon plants inoculated at transplanting with a mixture of races 1 and 2 of *F. oxysporum* f. sp. *niveum* and that developed symptoms of wilting in spring 2010 in a field site in Charleston, SC

Rootstock cultivar, treatment ^z	Recovery (%) of <i>F. oxysporum</i> ^y		
	Top of plant	Base of plant	Top and base of same plant
Tri-X 313, nongrafted	100	100	100
Tri-X 313 self-grafted	50	100	50
Ojakkyo grafted	67	83	67
Shintosa Camel grafted	33	33	33
Strong Tosa grafted	50	50	33
Macis grafted	17	83	17
Emphasis grafted	33	83	33
WMXP 3945 grafted	40	40	40
Mean percentage	48.9	72.3	46.8
Total	(n = 23)	(n = 34)	(n = 22)

^y Recovery was based on culturing symptomatic vines from 47 plants, six plants per cultivar, one plant from each field replication, except for WMXP 3945, which had symptomatic plants in only five replications when sampling was done on 28 April and 7 May 2010. Refer to the main text for details on the isolation method.

^z Refer to Table 1 for details of each cucurbit cultivar. Total = total number of plants yielding *F. oxysporum* isolates.

Table 4. Mean yield across experiments in spring 2010 and spring 2011 of seedless watermelon Tri-X 313 grafted onto six cucurbit rootstocks and control watermelon treatments in a field infested with *Fusarium oxysporum* f. sp. *niveum* in Charleston, SC^y

Rootstock cultivar, treatment ^z	Fruit weight (Mg/ha)	Fruit number/ha	Average fruit weight (kg)
Emphasis grafted	51.0 a	7,303 a	7.09 b
Macis grafted	48.8 ab	6,883 ab	7.15 b
WMXP 3945 grafted	47.6 ab	6,661 abc	7.28 b
Strong Tosa grafted	46.6 ab	6,219 abcd	7.53 ab
Shintosa Camel grafted	40.5 bc	5,511 cd	7.43 ab
Ojakkyo grafted	43.0 abc	5,555 cd	7.83 a
Tri-X 313, nongrafted	35.8 c	5356 cd	6.57 c
Tri-X 313 self-grafted	33.2 c	5179 d	6.45 c
Pooled standard error	3.7	480	0.18

^y Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

^z Refer to Table 1 for details of each cucurbit cultivar.

of fruit per plant and total weight of fruit but not the percentage of marketable fruit (44). Seeded watermelon grafted onto bottlegourd rootstocks generally produced larger fruit than watermelon grafted onto interspecific hybrid squash rootstocks or the nongrafted control watermelon plants; however, the difference was significant only with Skopje (43,44). In addition to the yield increase observed from grafting onto bottlegourd, one interspecific squash cultivar in this study, Strong Tosa, also increased marketable weight and fruit size but not fruit number. Similarly, in Spain, rootstocks of interspecific hybrid squash and *C. moschata* increased marketable weight of grafted seedless watermelon in soil severely infested with *Fusarium* spp. compared with the three control treatments of a watermelon rootstock, a bottle gourd rootstock, and nongrafted plants (33). The interspecific hybrid squash rootstock 'Shintoza' consistently increased fruit size compared with nongrafted seedless watermelon (33). However, in Turkey, grafting onto Strong Tosa did not increase number of fruit per plant or total yield (44).

The increased returns obtained with watermelon grafted onto bottlegourd rootstocks could help offset the increased costs of grafted transplants compared with conventional, nongrafted transplants (3,22). Nongrafted transplants cost \$0.21 each in South Carolina and, at standard plant populations of 4,940/ha in the southeastern United States, represent \$1,037 of the \$7,705/ha production costs (8). Grafted transplants are estimated to cost three times as much as nongrafted transplants, which is approximately \$0.63 apiece or \$3,112/ha if 4,940 transplants/ha are set (4). The increase in crop value of \$3,617/ha with a bottlegourd rootstock (based on the average price of \$0.27/kg for watermelon in South Carolina between 2009 and 2011) would cover the additional cost of grafted watermelon transplants, \$2,075/ha, and leave \$1,542/ha in additional returns above those expected from a nongrafted cultivar susceptible to *Fusarium* wilt grown in an infested field (8,40). In Israel, growers have been able to reduce the number of grafted watermelon plants transplanted per hectare due to increased fruit production on grafted compared with nongrafted plants; this is another option for improving the cost-benefit ratio of grafted watermelon (3).

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