Fruit Yield, Disease Incidence, and Root Colonization of Hybrid Muskmelons Resistant to Fusarium Wilt

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ABSTRACT

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Eight F_1 hybrid muskmelons, bred for resistance to race 2 of *Fusarium oxysporum* f. sp. *melonis*, and an open-pollinated cultivar susceptible to Fusarium wilt were grown in 1987 in a commercial muskmelon field naturally infested with *F. o.* f. sp. *melonis* race 2 and in a field where the pathogen was absent. In 1988, nine disease-resistant hybrids and a susceptible cultivar were evaluated in trials at both locations. In both years, a high incidence of Fusarium wilt was observed on the susceptible cultivar in the infested field. Either no disease or very low levels were recorded for the wilt-resistant entries grown in the naturally infested soil. Resistant entries also yielded more fruit when grown in infested soil than the susceptible cultivar. Many of the hybrids also produced superior yields in the absence of the disease. *F. o.* f. sp. *melonis* colonized seedling roots of susceptible and resistant cultivars to the same extent but was recovered significantly more often from roots of the susceptible cultivar when mature plants were sampled.

Fusarium wilt of muskmelon (*Cucumis melo* L.), caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *melonis* W. C. Snyder & H. N. Hans., was first reported in the San Joaquin Valley of California in 1976 (5). Since then it has become progressively more widespread, and it is now the most important soilborne disease affecting muskmelon production in California.

Effective control of this disease generally has been achieved only through the use of disease-resistant cultivars (11,19). For this reason, when Fusarium wilt became a problem in California, a breeding program was initiated to incorporate disease resistance into "western shipping-type" muskmelon germ plasm (16–19). Seed companies have since used this germ plasm to develop F_1 hybrids that are resistant to races 0 and 2 of *F. o.* f. sp. *melonis* and that are suitable for California growing conditions.

We evaluated the field performance of these disease-resistant cultivars and breeding lines at two locations in 1987 and 1988. One location was a commercial field naturally infested with race 2 of *F. o.* f. sp. *melonis*. Concurrent studies at this location established that the pathogen could be distinguished from nonpathogenic strains of *F. oxysporum* by its colony morphology on a selective medium (4). Using this criterion to identify the pathogen, we determined the frequency of root colonization of resistant and susceptible muskmelons by *F. o.* f. sp. *melonis*. We also evaluated fruit yield and disease incidence for all entries at both locations.

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MATERIALS AND METHODS

Field plots. In 1987, a trial was conducted in a commercial field on the west side of Fresno County in the San Joaquin Valley of California. In 1986, Fusarium wilt devastated a muskmelon crop grown in this field; the pathogen was identified as race 2 of *F. o.* f. sp. *melonis* (8).

We used soil dilution plating to estimate preplant soil inoculum densities of *F. o.* f. sp. *melonis* in the field (4). Soil populations in five samples taken from randomly selected locations within the field plot ranged from 350 to 500 propagules per gram of soil. *F. o.* f. sp. *melonis* was identified on soil dilution plates by its colony morphology, as described below under root colonization.

The 1987 trial included the hybrid cultivar Easy Rider (Harris-Moran Seed Co., Hayward, CA) and seven F_1 hybrid breeding lines: XPH-5087, XPH-5088, and XPH-5089 (Asgrow Seed Co., Kalamazoo, MI); PSX-6283, PSR-10885, and PSR-11185 (Petoseed Co., Woodland, CA); and NVH-879 (Northrup-King Seed Co., Woodland, CA). Muskmelon cultivar PMR 45 SJ (Asgrow Seed Co.), an open-pollinated cultivar susceptible to Fusarium wilt, was included as a control.

Each entry was seeded in five replicate plots 15 m long and 0.2 m wide in a randomized complete block design. The first planting of this experiment was on 5 May. On 27 May, the same entries were planted in the same experimental design adjacent to the first planting.

In the same year, the same nine cultivars and breeding lines were evaluated at the University of California West Side Field Station (WSFS) in Fresno County. Fusarium wilt of muskmelon has never been observed at WSFS, and *F. o.* f. sp. *melonis* was not detected by our soil dilution plate assay. The detection threshold for this assay was approximately 10 propagules per gram of soil. The experimental design at WSFS was the same as that used at the commercial field. Planting was on 5 April.

In 1988, a field trial was conducted at a different location within the same commercial field used in 1987. In this part of the field, which was planted to cotton in 1987, preplant inoculum densities of *F. o.* f. sp. *melonis* ranged from 450 to 600 propagules per gram of soil. Ten entries were included in a randomized complete block design with five replications. The susceptible cultivar PMR 45 SJ was included as a control, along with Easy Rider and eight F_1 hybrid breeding lines: XPH-5087; PSX-1983, PSX-2083, PSX-31787, PSR-32287, and PSR-32387 (Petoseed Co.); NVH-879; and Sunnex 7002 (Sun Seeds Co., Hollister, CA). Petoseed Co. has released PSX-1983 as commercial cultivar Durango. The two planting dates in 1988 were 1 May and 1 June.

A separate planting of Easy Rider and PMR 45 SJ was included in the commercial field in 1988 for root colonization studies. Each cultivar was seeded on 1 May in four replicate plots 12 m long and 0.2 m wide.

At WSFS the 1988 field trial was planted on 9 April in a randomized complete block design with five replications. All the entries planted in the commercial field were included in the WSFS trial except Easy Rider and Sunnex 7002.

Disease ratings. The healthy plants and the plants showing symptoms of Fusarium wilt were counted in each plot after thinning and again just before harvest. Seedlings affected by Fusarium wilt were identified by foliar symptoms, including mild to severe chlorosis of young leaves and necrosis at leaf margins. Infected seedlings often were stunted relative to healthy plants. Older plants had similar symptoms and were further characterized by a lesion on the

main stem near the soil line that extended distally from the crown of the plant. When necessary, isolations from plant tissue were made on Komada's selective medium (KM) (10).

Pathogenicity tests. Isolate pathogenicity was confirmed in greenhouse tests using a seedling root dip assay (8). In this assay, 7- to 10-day-old seedlings of the Fusarium wilt-susceptible muskmelon cultivar Top Mark were dipped in a suspension containing 2.5×10^5 spores of the isolate being tested per milliliter. The inoculated seedlings were replanted in a steamed soil mix (8).

A tray-inoculation procedure (13) was used to test F_1 hybrids for susceptibility to Fusarium wilt under greenhouse conditions at Davis, CA. In this procedure, a tray in which seed was planted was placed on top of a second tray filled with UC soil mix (12). About 2 wk after planting, when seedling roots had grown through drainage holes into the lower tray of soil mix, the upper tray was lifted off the lower tray and placed in a cafeteria tray filled with 1 L of a suspension containing 2.5×10^5 spores of race 2 of *F. o.* f. sp. *melonis* per milliliter. After the 1-L suspension was absorbed, the seedling tray was removed and was again placed on top of the tray containing UC soil mix.

Yield data. Mature fruit was harvested seven to nine times over a 3-wk period at each location in both years. Melons were harvested from 12 m of each 15-m plot. The marketable melons harvested from each plot were divided among four size classes corresponding to the number of melons required to fill one carton (12, 15, 18, or 23). From the number of melons in each size class, the corresponding number of cartons per plot was calculated. Yield data were expressed as the number of cartons per hectare.

Root colonization. Colonization of plant roots by *F. o.* f. sp. *melonis* was quantified for all entries included in the commercial field trial in 1987 except PSR-11185 and XPH-5089. No root samples were taken from WSFS trials in either year. In 1987, root samples were collected from soil cores taken near the center of the bed 5–10 cm from the crown of a plant. No samples were taken adjacent to plants showing symptoms of Fusarium wilt. Seven to 10 cores were taken from each of three of the five replications of each entry. Root samples were collected 8, 9, and 12 wk after seeding.

Roots were removed from soil cores, washed three times for 30 min in 1% sodium hexametaphosphate, given a final rinse with sterile distilled water, and placed in parallel rows on plates of KM. A minimum of 100 cm of roots was plated for each replicate. Plates were incubated for 6–7 days under fluorescent light for 12 hr per day.

Colonies of *F. oxysporum* identified on KM were of two morphological types. Those characterized by fluffy white mycelium and a reddish purple pigment in the agar were always pathogenic to muskmelon in seedling root dip assays (4). Those characterized by a beige color, minimal development of aerial mycelium, and abundant sporodochia on KM were always avirulent (4). Isolates with the colony morphology associated with the pathogen were always vegetatively compatible with our tester strains of *F. o.* f. sp. *melonis* (8,9), and those with the colony morphology associated with nonpathogens were always vegetatively incompatible with *F. o.* f. sp. *melonis* (8; T. R. Gordon, *unpublished*).

A root colonization study was also conducted under controlled conditions in 1987. Each of the seven breeding lines sampled in the field trial was grown in soil naturally infested with about 400 propagules of race 2 of *F. o.* f. sp. *melonis* per gram. The experiment included three replicate 150-ml pots for each entry, with three to five plants per pot. Plants were maintained in a growth chamber at day-night temperatures of 25 and 18 C, with a 12-hr photoperiod. Roots (200 cm) were sampled from each replicate 4 wk after seeding. Roots in the bottom 2 cm of the pot were excluded from the samples (4). Roots were washed and assayed as described for roots collected in the field.

In 1988, root colonization of PMR 45 SJ and Easy Rider in the commercial field was evaluated. Four randomly selected asymptomatic plants, along with as much of the root systems as possible, were taken from each of four replicate plots of each cultivar 4 and 10 wk after seeding. Roots (100 cm from each replicate) were washed and assayed as described for roots collected in 1987. In addition, a stem section was taken from each of the sampled plants at the cotyledonary node, rinsed with running water, immersed for 10 sec in 70% ethanol and then for 1.5 min in 0.5% sodium hypochlorite, and placed on KM. Petioles taken 10–20 cm distal to the cotyledonary node were treated in the same manner. Stem and petiole isolation plates were incubated as described for root isolation plates. After 7 days, tissue pieces were scored for the presence or absence of *F. o.* f. sp. *melonis*.

Data analysis. Analysis of variance (ANOVA) was used to evaluate the significance of differences in fruit yield and in root colonization by *F. o.* f. sp. *melonis*. For root colonization data collected from the same plot at different times in 1987, repeated-measures ANOVA was used. Means were compared using Duncan's multiple range test. ANOVA was performed and means were compared using log-transformed data; untransformed data are reported. Statis-

Table 1. Disease incidence (percentage of plants showing symptoms of Fusarium wilt) in hybrid muskmelons in field and greenhouse tests

Year Cultivar[a]	Field test[b] 5 May planting	Field test[b] 27 May planting	Greenhouse test[c]
1987			
PMR 45 SJ	84.0	68.0	100.0
XPH-5088	1.6	0.0	0.0
XPH-5089	0.0	0.5	0.0
XPH-5087	1.2	0.5	0.0
Easy Rider	0.0	0.6	0.0
NVH-879	0.0	0.0	11.0
PSX-6283	0.3	0.0	0.0
PSR-10885	0.0	0.3	0.0
PSR-11185	0.9	0.0	3.8
1988			
PMR 45 SJ	66.0	53.5	100.0
XPH-5087	0.0	0.0	0.0
Easy Rider	0.0	0.0	0.0
NVH-879	0.0	0.3	0.0
PSX-1983 (Durango)	0.0	0.0	0.0
PSX-2083	0.0	0.0	0.0
PSX-31787	0.0	0.0	0.0
PSR-32287	0.4	0.0	0.0
PSR-32387	0.0	0.0	0.0
Sunnex 7002	0.0	0.0	5.9

[a] PMR 45 SJ is an open-pollinated cultivar susceptible to Fusarium wilt. Easy Rider and all other entries are F_1 hybrids with resistance to Fusarium wilt.

[b] Data are means of five replicates. Each replicate was a plot 15 m long and 0.2 m wide. Plants were rated for disease after thinning and just before harvest. A minimum of 110 plants were rated for each entry in each planting date. Plots were located in a commercial field on the west side of Fresno County in the San Joaquin Valley. Soil in this field was naturally infested with approximately 400 and 500 propagules of *Fusarium oxysporum* f. sp. *melonis* race 2 per gram in 1987 and 1988, respectively.

[c] A tray-inoculation procedure was used, with a spore suspension of *F. o.* f. sp. *melonis* race 2 at a concentration of 250,000 spores per milliliter. At least 16 plants were tested for each entry.

tical computations were performed with either NCSS version 5.0 (J. L. Hintze, Kaysville, UT) or SAS version 5.18 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Trials in the commercial field in both 1987 and 1988 showed a high incidence of Fusarium wilt in the susceptible cultivar PMR 45 SJ (Table 1). Disease incidence was very low for Easy Rider and all the hybrid breeding lines (Table 1). The low levels of disease observed in 1987 in some plots seeded to a resistant entry might have arisen from the growth of seed from the susceptible cultivar grown the previous year. However, because these plants died before reaching maturity, fruit characteristics could not be used to confirm their identity. Fewer diseased plants were identified among the resistant entries in the part of the field used in 1988, where the preceding crop was cotton.

Some of the breeding lines may also have included a small percentage of susceptible individuals. This possibility seems likely for NVH-879, PSR-11185, and Sunnex 7002, all of which showed some disease in greenhouse tests (Table 1). This could have resulted from a simple mechanical mixture of seed or, if the female parent of the cross was susceptible, from self-pollination resulting from a failure to effectively emasculate all the hermaphroditic flowers of the female parent.

In 1988, *F. o.* f. sp. *melonis* was isolated from four diseased plants within plots seeded to resistant entries. These four isolates were tested for pathogenicity on differential cultivars to determine their race (14). All four were virulent on Top Mark and CM 17-187 and avirulent on Perlita FR, indicating that they belonged to race 2. Thus there was no indication that race 1 of *F. o.* f. sp. *melonis* was present in our field plot. This is consistent with our previous observations that only race 2 of *F. o.* f. sp. *melonis* occurs in the San Joaquin Valley of California (8; F. W. Zink, *unpublished*).

In the commercial field trial (infested soil) in 1987, the yield of PMR 45 SJ was only 30% of the mean yield for the F_1 hybrids, reflecting the impact of Fusarium wilt on the susceptible cultivar (Table 2). In contrast, at WSFS (noninfested soil), the yield of PMR 45 SJ was 91% of the mean yield of the F_1 hybrids. Similarly, in 1988, PMR 45 SJ produced 44% of the mean yield of the resistant entries in infested soil, compared with 80% in noninfested soil (Table 2).

In both years, the average yield of the resistant hybrids was higher at WSFS than at the commercial field. Lower yields at the commercial site may have been the result of the presence of *F. o.* f. sp. *melonis;* however, numerous other factors may also have contributed to the observed differences in yield. For example, water management was more effective at WSFS, where the plots were on nearly level ground, than at the commercial field, where irrigation runs were relatively steep. Because we cannot determine the relative importance of this and other biotic and abiotic differences between the two locations, we cannot conclude that *F. o.* f. sp. *melonis* had a significant effect on the yield of the resistant entries.

In addition to their disease resistance and high yields, many of the hybrids produced fruit with commercially desirable characteristics. Easy Rider, PSX-31787, and PSX-1983 (Durango) all had sutureless fruit with good netting, thick flesh, and high soluble solids (13.2–13.4%). NVH-879 also produced commercially acceptable melons, but fruit quality was less uniform.

In the field in 1987, *F. o.* f. sp. *melonis* was recovered significantly more often from roots of PMR 45 SJ than from roots of the disease-resistant entries on all three sampling dates (P=0.05) (Table 3). Because there were no significant time × treatment interactions, data from all three sampling dates were combined. In contrast, our growth chamber experiment revealed no significant differences in the frequency with which *F. o.* f. sp. *melonis* colonized the roots of PMR 45

Table 2. Fruit yield (cartons per hectare) of hybrid muskmelons grown in field plots infested or not infested with *Fusarium oxysporum* f. sp. *melonis* race 2[a]

Year Cultivar[b]	Infested soil[c] 5 May planting	Infested soil[c] 27 May planting	Noninfested soil[d]
1987			
PMR 45 SJ	617 c	442 c	2,537 bc
XPH-5088	2,087 a	1,657 a	3,258 a
XPH-5089	2,393 a	1,764 a	3,485 a
XPH-5087	2,505 a	1,741 a	3,520 a
Easy Rider	1,924 ab	1,504 ab	2,692 b
NVH-879	1,561 bc	1,242 ab	1,907 d
PSX-6283	2,270 a	1,576 a	3,189 a
PSR-10885	1,803 bc	1,230 ab	2,228 bc
PSR-11185	1,880 bc	904 bc	2,028 bc
1988			
PMR 45 SJ	934 b	963 c	2,282 c
XPH-5087	1,860 a	2,223 b	2,996 ab
Easy Rider	1,892 a	2,048 b	—[e]
NVH-879	1,929 a	2,216 b	3,369 a
PSX-1983 (Durango)	2,018 a	2,379 b	2,796 b
PSX-2083	1,877 a	2,213 b	2,665 bc
PSX-31787	2,033 a	1,857 b	2,880 b
PSR-32287	2,332 a	2,134 b	2,621 bc
PSR-32387	2,430 a	2,934 a	2,766 b
Sunnex 7002	1,857 a	2,206 b	—[e]

[a] Data are means of five replicates. Means in the same column followed by a common letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test. The marketable melons harvested from each plot were divided among four size classes. The number of cartons was calculated from the number of melons in each size class. Cartons weigh about 18 kg.
[b] PMR 45 SJ is an open-pollinated cultivar susceptible to Fusarium wilt. Easy Rider and all other entries are F_1 hybrids with resistance to Fusarium wilt.
[c] Plots were located in a commercial field on the west side of Fresno County in the San Joaquin Valley. Soil in this field was naturally infested with approximately 400 and 500 propagules of *F. o.* f. sp. *melonis* race 2 per gram in 1987 and 1988, respectively.
[d] Plots were located at the University of California West Side Field Station in Fresno County; planting was on 5 April.
[e] Not tested.

Table 3. Colonization of hybrid muskmelon roots by *Fusarium oxysporum* f. sp. *melonis* in 1987 field trials and growth chamber experiments

Cultivar[a]	Colonies per 100 cm of root[b] Field[c]	Colonies per 100 cm of root[b] Growth chamber[d]
PMR 45 SJ	161 b	59 a
XPH-5088	50 a	56 a
XPH-5087	9 a	63 a
Easy Rider	13 a	67 a
NVH-879	14 a	77 a
PSX-6283	12 a	53 a
PSR-10885	11 a	59 a

[a] PMR 45 SJ is an open-pollinated commercial cultivar susceptible to Fusarium wilt. Easy Rider and all other entries are F_1 hybrids with resistance to Fusarium wilt.
[b] Means in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[c] Data are means from samples collected at three times; on each occasion, samples were collected from three replicate plots. Plots were located in a commercial field on the west side of Fresno County in the San Joaquin Valley. Soil in this field was naturally infested with approximately 400 propagules of *F. o.* f. sp. *melonis* race 2 per gram. Each replicate included a minimum of 100 cm of roots.
[d] Data are means of three replicates; each replicate included 200 cm of roots.

SJ and any of the Fusarium wilt-resistant hybrids (P=0.05) (Table 3). We conducted a second growth chamber experiment using PMR 45 SJ, Easy Rider, and PSX-6283 and two additional experiments using PMR 45 SJ and Easy Rider. In each experiment, we found no significant difference in the frequency with which *F. o.* f. sp. *melonis* was isolated from the roots of resistant and susceptible genotypes (P=0.05).

The discrepancy between the results of field and growth chamber experiments might be related to differences in age of the plants sampled or to differences between growth chamber and field-grown plants. Results from our 1988 study of root colonization in the field suggested that age was an important factor. Root colonization of seedlings (4 wk after seeding) of PMR 45 SJ and Easy Rider by *F. o.* f. sp. *melonis* did not differ significantly (10.5 and 16.0 colonies per 100 cm of roots, respectively) (P=0.05). At 10 wk after seeding (about 3 wk before harvest), however, *F. o.* f. sp. *melonis* was isolated at a significantly higher frequency from roots of PMR 45 SJ than from roots of Easy Rider (37.5 and 19.0 colonies per 100 cm, respectively) (P = 0.05).

We assume that colonization data for seedlings were primarily a measure of infection frequency (4,6,7). Thus we conclude, based on data from field and growth chamber experiments, that infection frequency by *F. o.* f. sp. *melonis* was not influenced by the resistance or susceptibility of the host. This conclusion is consistent with our previous work showing relatively little specificity in root colonization of nonsusceptible crops by *F. o.* f. sp. *melonis* (4).

As melon plants approach maturity in the field, susceptible cultivars may become more prone to infection than resistant genotypes. Alternatively, the pathogen may grow beyond the initial point of infection on roots of susceptible plants. If such secondary growth is more limited on roots of resistant plants (1), then this could explain our results, in both 1987 and 1988, showing significantly more colonies per centimeter of root on mature plants of the susceptible cultivar.

Isolations from the cotyledonary node of 4-wk-old plants of PMR 45 SJ and Easy Rider taken for root samples showed no infection by *F. o.* f. sp. *melonis*. However, the pathogen was recovered from the cotyledonary node and at least one petiole of 44% of the PMR 45 SJ plants sampled at 10 wk. Thus, the pathogen apparently colonized the vascular tissue in the stems of these plants, even though no external symptoms were evident. *F. o.* f. sp. *melonis* was never isolated from cotyledonary nodes or petioles of Easy Rider. A few roots of Easy Rider (10-wk-old plants) gave rise to numerous contiguous colonies of *F. o.* f. sp. *melonis* on KM, suggesting that these roots may have been systemically infected. This pattern of pathogen growth was more commonly observed on roots of PMR 45 SJ.

The source of the genetic resistance in the F_1 hybrids included in this study was either muskmelon cultivar Doublon (*Fom*-1) or Perlita FR (*Fom*-3) (15–18). Our results indicate that differences in genetic background had no significant effect on the effectiveness of these genes. Our results also confirm the utility of greenhouse seedling assays in predicting the efficacy of genetic resistance of muskmelon to Fusarium wilt under field conditions.

The resistance conferred by *Fom*-1 or *Fom*-3 apparently does not influence the frequency of root infections by *F. o.* f. sp. *melonis*. Resistance does appear to correlate with a lack of vascular colonization at or above the cotyledonary node under field conditions. However, because resistance is effective in a seedling root dip test, which should facilitate pathogen entry into the vascular system, exclusion of the pathogen from the xylem is unlikely to be solely responsible for Fusarium wilt resistance in muskmelon. It is more likely that a resistant genotype minimizes the spread of those infections that do reach the xylem, as has been described for resistance to Fusarium wilt in tomato (1,3).

The availability of cultivars with resistance to Fusarium wilt should eliminate a significant constraint on muskmelon production in the San Joaquin Valley of California. If race 1 is introduced into the state, however, continued control of Fusarium wilt would require incorporation of *Fom*-2 into a suitable horticultural type (14). Race 1 is found in Europe and recently was reported in Maryland (2). Race 1,2 would pose a more serious problem because there is no known source of resistance to this race of *F. o.* f. sp. *melonis*. Race 1,2 occurs in Europe but has not yet been identified in North America.

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