Effects of Inoculum Concentration on the Apparent Resistance of Watermelons to *Fusarium oxysporum* f. sp. *niveum*

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ABSTRACT

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The apparent wilt resistance of watermelon (*Citrullus lanatus*) cultivars to *Fusarium oxysporum* f. sp. *niveum* was dependent on the concentration of initial inoculum. Four wilt resistance rankings were established: susceptible (>80% wilt), slightly resistant (51–80% wilt), moderately resistant (21–50% wilt), and highly resistant (≤20% wilt). Wilt resistance rankings for most cultivars usually dropped one level as inoculum concentration increased logarithmically. Two cultivars, Dixielee and Smokylee, remained highly resistant up to inoculum levels of 1×10^6 conidia per milliliter. Populations of *F. oxysporum* f. sp. *niveum* recovered from soil indicated that planting wilt-susceptible cultivars tends to increase the pathogen population in the soil, whereas planting cultivars highly resistant to wilt decreases it.

Fusarium oxysporum f. sp. *niveum* (E. F. Sm.) Snyd. & Hans., causal agent of Fusarium wilt of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), occurs throughout the watermelon-growing regions of the world and in many cases is the limiting factor in watermelon production. Once the pathogen has infested a field, the fungus may survive in the soil for many years. Control of this disease has been primarily through virgin land plantings, long-term crop rotations, and use of Fusarium wilt-resistant cultivars (4,11).

Several authors have evaluated watermelon cultivars for wilt resistance (1,2,4,6,9–12). In spite of reports indicating a gradation in wilt resistance from none to very high, most cultivars are described simply as resistant or susceptible to Fusarium wilt and the degree of resistance is not usually indicated (1,4,11). These gradations in resistance levels among cultivars pose potential problems for farmers and extension agents to know which cultivar to grow or recommend.

In addition to differences in wilt resistance levels among cultivars, wilt resistance may also be affected by population levels of the pathogen. Douglas (3) reported that wilt severity of two muskmelon cultivars decreased with a curvilinear relationship to the log concentration of *F. oxysporum* f. sp. *melonis* propagules. He emphasized the need for a range of inoculum concentrations in testing new breeding material.

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The purpose of our study was to determine the effects of increasing inoculum pressure on the wilt resistance of some commercially available watermelon cultivars and the effect wilt-resistant or -susceptible cultivars have on soil populations of the fungus. An abstract of this work has been published (8).

MATERIALS AND METHODS

Test plants and inoculation. Seeds of 17 watermelon cultivars were purchased from several commercial sources (Willhite Melons, Weatherford, TX; Twilley Seed Co., Trevose, PA; Asgrow Seed Co., Kalamazoo, MI; Ferry Morse Seed Co., Mountain View, CA; and Niagra Seeds, Modesto, CA). Included among these were representative cultivars rated susceptible, slightly resistant, moderately resistant, or highly resistant to Fusarium wilt (4).

About 250 seeds of each cultivar were planted in flats containing a sandy loam:peat:perlite soil mix (3:3:1, v/v) and germinated. When the first true leaf was evident (about 2 wk after planting), the seedlings were uprooted and the roots washed under a stream of gently flowing water. One hundred eighty seedlings of each cultivar were selected and separated into six groups of 30 seedlings each. The six seedling groups were inoculated by dipping roots of each into a suspension of *F. oxysporum* f. sp. *niveum* (ATCC 18476) conidia adjusted to one of the following concentrations: 0 (diluted sterile medium), 1×10^3, 1×10^4, 1×10^5, or 1×10^6 conidia per milliliter. The inoculum was prepared by filtering a liquid broth culture through eight layers of cheesecloth and adjusting the predominantly microconidial suspension to appropriate concentrations with the aid of a Spencer hemacytometer. The inoculated seedlings were transplanted into 3.8-L plastic pots (five seedlings per pot) containing sandy loam:perlite (3:1, v/v) and maintained in the greenhouse. There were six replicated pots of each inoculum concentration for each of the 17 cultivars. Observations for symptoms of disease were made daily for 3 wk. The experiment was conducted over a 4-mo period from September to December 1981.

Cultivars were ranked for Fusarium wilt resistance based on the amount of wilt expressed after 3 wk. Four categories were established: highly resistant (≤20% wilt), moderately resistant (21–50% wilt), slightly resistant (51–80% wilt), and susceptible (>80% wilt). These categories closely follow the significant separations of percent wilt at $P = 0.05$ and are similar to previously established categories (4).

Recovery of *F. oxysporum* f. sp. *niveum* from soil. At the end of the test, soil populations of *F. oxysporum* f. sp. *niveum* were determined for each inoculum density treatment for eight cultivars. Any remaining plant material was removed from the pots and the soil from all six replicated treatments was combined in a 19-L bucket. After thorough mixing, about 30 g of soil was collected for analysis. A 1-g air-dried sample was placed into a flask containing 99 ml of sterile distilled water and shaken for 10 min. One milliliter of this suspension was plated on Komada's selective medium (7) and the remainder was diluted 1:10 and 1:100. One milliliter of these dilutions (10^{-3} and 10^{-4}) was also plated on Komada's medium. There were two replicated plates for each dilution. All plates were incubated at 22 C for 10 days. Colonies typical of *F. oxysporum* were counted and averaged among the replicates and a population density was calculated for each treatment.

Pathogenicity of isolates recovered from soil. Four *F. oxysporum* isolates recovered from soil dilution plates (designated soil isolates A, B, C, and D) were tested for pathogenicity to watermelon. The isolates were grown in liquid-shake culture; the conidia were filtered through cheesecloth and adjusted to 1×10^6 conidia per milliliter. Two-week-old Black Diamond watermelon seedlings were root-dipped (10 seedlings per isolate) in this inoculum and transplanted.

Percent wilt caused by each isolate was recorded after 2 wk.

RESULTS

In all but three cultivars tested, the wilt resistance rankings were affected by differences in inoculum levels (Tables 1 and 2). Most cultivars expressed little disease at the low inoculum level (10^3 conidia per milliliter) and were rated either moderately or highly wilt resistant. When the inoculum level was increased 10-fold, most dropped a rank in rating. Exceptions to this were the cultivar Black Diamond, rated susceptible at all inoculum levels, and cultivars Dixielee and Smokylee, rated highly resistant at each inoculum level tested.

After 3 wk at the lowest inoculum level (10^3 conidia per milliliter), eight cultivars were highly resistant, four were moderately resistant, four were slightly resistant, and only one was susceptible to wilt. As the inoculum level increased, significant shifts occurred in the rankings. At 10^4 conidia per milliliter, there was a 50% reduction in the number of highly resistant cultivars and seven were rated as wilt susceptible. At the highest inoculum level (10^6 conidia per milliliter), only Dixielee and Smokylee had less than 20% wilt and were rated highly wilt resistant. Calhoun Gray and Crimson Sweet were highly wilt resistant at low and intermediate inoculum levels, but resistance decreased at the high inoculum levels (10^5 and 10^6 conidia per milliliter, respectively). Charleston 76 and Jubilee, both popular cultivars, showed little resistance to wilt with increased inoculum pressure. The trend of decreased wilt resistance with increased inoculum pressure was the same for results at both 2 and 3 wk, but it was more evident at 3 wk.

When the cultivars were analyzed separately for percentage wilt at each inoculum concentration, four groups were established (Table 2). The first group was indicative of highly susceptible cultivars. There was generally a difference in wilt resistance between the two lowest inoculum levels, but maximum susceptibility was reached at 10^4 conidia per milliliter and increasing inoculum thereafter did not change the rating. This group included the first eight cultivars. The second group included three cultivars. These were highly wilt resistant at the low inoculum level but rapidly lost that resistance with a 10-fold increase in inoculum. The third group included four cultivars that showed a gradation in wilt resistance with increasing inoculum pressure. As inoculum increased, resistance decreased until maximum wilt was expressed for that particular cultivar. Cultivar Summit demonstrated this particularly well as resistance ratings changed at each inoculum level. The last group included cultivars Dixielee and Smokylee. These were rated highly resistant to Fusarium wilt at each inoculum level tested and were the only cultivars to be so throughout the inoculum range used.

Table 1. Percentage wilt of watermelon cultivars 3 wk after inoculation with increasing concentrations of *Fusarium oxysporum* f. sp. *niveum* conidia

Cultivar	Inoculum level (conidia/ml)[z]				
	0	10^3	10^4	10^5	10^6
Black Diamond	6.6 a	83.3 a	100 a	100 a	86.6 ab
Tendersweet	0.0 a	76.6 a	96.6 a	100 a	100 ab
Jubilee	3.3 a	66.6 ab	96.6 a	100 a	100 a
Sugarbaby	0.0 a	63.3 ab	76.6 bcd	96.6 a	93.3 ab
Mountain Sweet	0.0 a	60.0 abc	100 a	100 a	100 ab
Congo	14.3 ab	41.4 bcd	93.1 ab	100 a	100 a
Garrisonian	20.0 b	26.0 de	85.0 bc	100 a	100 a
White-Seeded Watson	13.3 ab	43.3 bcd	73.3 cd	100 a	93.3 ab
Sunshade	0.0 a	5.2 ef	95.0 ab	62.0 c	90.0 ab
Tendergold	10.3 ab	10.3 ef	73.3 cd	92.9 ab	93.3 ab
Peacock	0.0 a	13.3 ef	60.0 de	85.7 ab	96.6 ab
Charleston 76	0.0 a	36.6 cd	50.0 e	73.3 bc	100 a
Summit	0.0 a	0.0 f	23.3 f	60.0 c	80.0 b
Calhoun Gray	0.0 a	0.0 f	3.3 g	23.3 d	50.0 c
Crimson Sweet	0.0 a	0.0 f	0.0 g	14.3 de	20.7 d
Dixielee	0.0 a	3.3 ef	0.0 g	10.0 de	13.3 de
Smokylee	0.0 a	0.0 f	0.0 g	3.3 e	3.3 e

[z] Numbers followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Wilt resistance ratings of watermelon cultivars inoculated with increasing concentrations of *Fusarium oxysporum* f. sp. *niveum* conidia

Cultivar resistance rating[a]	Inoculum level (conidia/ml)			
	10^3	10^4	10^5	10^6
Group 1[b]				
Black Diamond	+	+	+	+
Tendersweet	SR	+	+	+
Jubilee	SR	+	+	+
Sugarbaby	SR	SR	+	+
Mountain Sweet	SR	+	+	+
Congo	MR	+	+	+
Garrisonian	MR	+	+	+
White-seeded Watson	MR	SR	+	+
Group 2				
Sunshade	HR	+	SR	+
Tendergold	HR	SR	+	+
Peacock	HR	SR	+	+
Group 3				
Charleston 76	MR	MR	SR	+
Summit	HR	MR	SR	+
Calhoun Gray	HR	HR	MR	MR
Crimson Sweet	HR	HR	HR	MR
Group 4				
Dixielee	HR	HR	HR	HR
Smokylee	HR	HR	HR	HR

[a] Based on percentage wilt 3 wk postinoculation. HR = highly resistant (<20% wilt), MR = moderately resistant (21–50% wilt), SR = slightly resistant (51–80% wilt), and + = highly susceptible (≥80% wilt).

[b] Cultivars reacting similarly to increasing inoculum levels.

Recovery of *F. oxysporum* f. sp. *niveum* from soil. Soil populations of *F. oxysporum* f. sp. *niveum* corresponded reasonably well with inoculum levels used (Table 3). Two trends were observed: First, higher populations of *F. oxysporum* f. sp. *niveum* propagules were recovered from soil as inoculum levels increased. Second, as wilt resistance of the cultivars increased, fewer *F. oxysporum* f. sp. *niveum* propagules were recovered from soil. No *F. oxysporum*-type colony was recovered from the Dixielee soil at any level. This was somewhat surprising because there was some wilt observed in this cultivar at the higher inoculum levels (Table 1). Likewise, *F. oxysporum* f. sp. *niveum* was not recovered from the Summit soil at any inoculum level except 10^6 conidia per milliliter even though significant ($P = 0.05$) wilt occurred at 10^4 and 10^5 conidia per milliliter (Table 1). Failure to isolate the fungus from the Dixielee and Summit soils probably reflects sampling errors as opposed to any special trend.

Pathogenicity of isolates recovered

from soil. All four isolates recovered from soil were highly pathogenic to Black Diamond watermelon. "Soil isolate A" and "soil isolate B" caused wilt in 90% of the inoculated seedlings within 2 wk. Soil isolate C" and "soil isolate D" both caused 100% wilt and death of the transplants within 2 wk.

Table 3. Population levels of *Fusarium oxysporum* f. sp. *niveum* recovered from soil 2 wk after inoculation of watermelon cultivars

Cultivar	Average propagules recovered/gram of soil[a] according to initial inoculum concentration (conidia/ml)				
	0	10^3	10^4	10^5	10^6
Black Diamond	0	300	400	4,200	900,000
Jubilee	0	200	2,600	4,200	22,000
White-seeded Watson	50	0	3,200	4,000	10,500
Charleston 76	0	0	2,500	1,500	2,300
Summit	0	0	0	0	8,000
Calhoun Gray	0	0	0	500	1,800
Crimson Sweet	0	200	100	100	400
Dixielee	0	0	0	0	0

[a] Counts based on dilution plates yielding 10–100 colonies.

DISCUSSION

The results of this study correspond closely with wilt resistance rankings of watermelon cultivars by other authors (1,3,11,12). It is also evident from this study that wilt resistance rankings are clearly affected by the concentration of pathogen propagules and are subject to change. This is potentially of major significance to the farmer. Elmstrom and Hopkins (4) demonstrated almost a fivefold reduction in yield of Charleston 76 when grown in a field that had been continually cropped to watermelon for 6 yr. Assuming a high population of the wilt *Fusarium* propagules in their fields, this finding correlates with the results of our study. Charleston 76 ranked highly or moderately wilt resistant at low inoculum levels but only slightly resistant or susceptible at higher inoculum levels. As noted earlier, decreased resistance to Fusarium wilt with increased inoculum pressure was also reported by Douglas (3) on muskmelons (*Cucumis melo* L. var. *reticulatus* Naud.).

In our study, only four cultivars maintained a moderate to high level of wilt resistance at high inoculum levels: Calhoun Gray, Crimson Sweet, Dixielee, and Smokylee. Three of these were included in Elmstrom and Hopkin's test (4) and each produced yields of >30 metric tons per hectare on fields continually cropped to watermelons. Of these, only Crimson Sweet is grown commercially to any extent in Texas.

Data on populations of *F. oxysporum* f. sp. *niveum* recovered from soil provides additional information on the buildup of the fungus in soil. Although the actual numbers of propagules may not directly relate to field data, the overall trend probably does. That is, planting wilt-susceptible or only slightly resistant cultivars provides for the rapid buildup of inoculum. On the other hand, highly wilt-resistant cultivars tend to decrease the pathogen population. Similar results were obtained by Wensley and McKeen (13) with *F. oxysporum* f. sp. *melonis* (which causes wilt in muskmelons.) They reported soil populations of the pathogen at harvest were at least seven times greater at plant sites in the presence of a susceptible host than at sites in the presence of a resistant cultivar.

Hine (5) reported that *F. oxysporum* f. sp. *niveum* grew rapidly in sterile soil and sporulated profusely. In unsterile soil, the microconidia germinated, the mycelium lysed, and the fungus was unrecoverable after short periods of incubation. This may explain why the pathogen was not recovered from the soil in which Summit, Calhoun Gray, and Dixielee were grown in our tests at certain inoculum levels even though wilt was observed before sampling. The soil used in this study was heat-pasteurized (68 C), not autoclaved, and was therefore not sterile. Second, in most instances, the soil was not used immediately but rather after being set aside for 1–2 wk. This allowed some microflora to become reestablished. The combination of "clean" but unsterile soil and cultivars moderately resistant to wilt would provide poor conditions for saprophytic survival of the fungus.

The use of wilt-resistant cultivars and long-term rotations continues to be the only means of controlling Fusarium wilt of watermelon. The use of cultivars highly resistant to wilt, such as Dixielee and Smokylee, may keep land more productive for watermelons for longer periods of time.

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