# Susceptibility of Summer Squash to the Watermelon Wilt Pathogen (*Fusarium oxysporum* f. sp. *niveum*)

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**ABSTRACT**

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Thirty-four cultivars encompassing six species of *Cucurbita* were tested for susceptibility to *Fusarium oxysporum* f. sp. *niveum*. All were resistant except 10 cultivars or hybrid accessions of *Cucurbita pepo* var. *melopepo* that include zucchini and yellow summer squashes. Percentage wilt in the susceptible cultivars ranged from 37 to 100%. Golden Eagle, Hyrific, Straightneck, and Early Prolific Straightneck were the most susceptible. Yellow Crookneck was resistant to *F. oxysporum* f. sp. *niveum* as were bush scallop, cushaw, and pumpkin. Resistance of certain cultivars to *F. oxysporum* f. sp. *niveum* was attributed to a reduced level of colonization in the vascular tissue rather than lack of penetration by the fungus.

Additional key words: *Citrullus lanatus*

Formae speciales of *Fusarium oxysporum* Schlecht., causal agents of many vascular wilt diseases, are generally noted for their host specificity. Their narrow host range was the primary impetus for Snyder and Hansen's revision of the *Fusarium* section Elgeans in 1940 (16). In their revision, they reduced 10 species to one and erected 25 formae speciales based on pathogenicity to particular hosts. Although not all wilt fusaria are host-specific, the formae speciales concept is a very useful system and has gained wide acceptance. Taubenhaus (19) concluded that the watermelon wilt *Fusarium* was pathogenic only to watermelons (*Citrullus lanatus*) and not to other cucurbitaceous or noncucurbitaceous species. Other sources indicated a wider host range (1,2,21) of the fungus, including the winter and summer squashes (*Cucurbita* spp.). Taubenhaus (19) specifically indicated that squash was not a susceptible host.

The most extensive study on the host range of the cucurbitaceous wilt fusaria was done by Armstrong and Armstrong (4). Over 30 years, they tested 109 isolates and races of cucurbit wilt fusaria on 90 cucurbit cultivars and plant introductions in a myriad of combinations. Included in these tests were 17 isolates of *F. oxysporum* f. sp. *niveum* cross-inoculated onto 17 watermelon cultivars, a citron, and a paddy melon (*Citrullus colocynthus*), and numerous muskmelons, cucumbers, and gourds. Their results showed that *F. oxysporum* f. sp. *niveum* was pathogenic only to watermelon and the closely related paddy melon.

Detailed as their studies were, they did not include the summer squashes (*Cucurbita pepo* var. *melopepo* Alef.) and thus failed to help resolve the discrepancy between Taubenhaus's work (19) and reports in the plant disease host indices (1,2,21).

Preliminary results in our laboratory suggested that summer squash was susceptible to the watermelon wilt *Fusarium*. The purpose of our study was to investigate further the pathogenicity of

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*F. oxysporum* f. sp. *niveum* to zucchini and yellow summer squashes.

## MATERIALS AND METHODS

**Test plants.** Twenty-nine cultivars and/or hybrid accessions of summer squash (*Cucurbita pepo* var. *melopepo*) were tested for susceptibility to *F. oxysporum* f. sp. *niveum* (Table 1). These included crookneck and straightneck yellow-fruit types, zucchini, and scallop fruit types. Five species of winter squash were also tested: a field pumpkin (*Cucurbita pepo* var. *pepo* (L.)), a butternut (*Cucurbita moschata* (Duchesne) Poir.), a hubbard (*Cucurbita maxima* Duchesne), a cushaw (*Cucurbita mixta* Pang.), and a gourd (*Cucurbita ficifolia* Brouche) type.

**Inoculation.** Five seeds of each cultivar or accession were planted in 1-gal plastic pots containing a heat-pasteurized sand:peat:perlite soil mix (3:3:1, v/v) infested with a microconidial suspension of *F. oxysporum* f. sp. *niveum* (ATCC #18467) at the rate of $6.25 \times 10^5$ spores per liter of soil. There were 10 replicate pots for each cultivar and four replicate pots for each accession. The inoculum was prepared by seeding 30 ml of a liquid mineral salts medium (8) in 125-ml Erlenmeyer flasks with a 3-mm plug from a potato-dextrose agar (PDA) culture. The cultures were incubated on a rotary shaker at 100 rpm at 27 C for 4 days under a 12-hr light/dark cycle. After incubation, the inoculum was aseptically strained through eight layers of cheesecloth. The filtrate was virtually all microconidia and was adjusted to the appropriate concentration with the aid of a Spencer hemacytometer.

Controls consisted of squash planted in uninfested soil and the susceptible watermelon Black Diamond planted in both infested and uninfested soil. Pots were maintained in or outside the greenhouse and monitored for disease development for 30 days.

**Light microscopy.** Two weeks after inoculation, root and stem tissue from representative plants of susceptible Early Prolific Straightneck and resistant Crookneck were fixed in formalin acetic acid, passed through a graded tert-butyl alcohol series and embedded in Paraplast (9). Serial sections 12-$\mu$m thick were cut on a rotary microtome and stained with safranin-fast green (9).

**Recovery of the pathogen from infected plants and proof of pathogenicity.** Representative samples of plants showing symptoms of wilt were removed and washed with tap water to remove soil particles. Stems and roots were separated, surface-disinfested for 10 min in 10% Clorox, and rinsed twice in sterile distilled water. Sections (1–3 mm) were cut with a razor blade and plated onto Komada's selective medium (10). Emergent colonies were transferred to PDA plates and incubated 5–7 days at room temperature. After incubation, a 3-mm plug from the colony periphery was removed and placed into the liquid mineral salts medium as described previously. The inoculum was adjusted to $1 \times 10^5$ spores per milliliter and used to inoculate 2-wk-old wilt-susceptible Tendergold watermelon seedlings by root dipping. Twenty-five seedlings were inoculated with each isolate. After inoculation, the seedlings were transplanted into 1-gal plastic pots (five seedlings per pot) containing a sandy-loam soil and perlite mix (3:1, v/v). Checks included 25 seedlings dipped in sterile, diluted medium and 25 seedlings inoculated with *F. oxysporum* f. sp. *niveum* (ATCC #18467).

**Table 1.** Percentage wilt of squash cultivars and accessions planted in soil infested with *Fusarium oxysporum* f. sp. *niveum*

| Species | Cultivar/accession | Fruit type[y] | Percent wilt[z] |
|---|---|---|---|
| *Citrullus lanatus* | Black Diamond | WM | 100.0 a |
| *Cucurbita pepo* | | | |
| var. *melopepo* | Golden Eagle | Y | 100.0 a |
| | S9-3753 | Z | 100.0 a |
| | S11-3756 | Z | 92.6 ab |
| | Hyrific | Y | 88.6 ab |
| | S10-3754 | Z | 77.4 bc |
| | Straightneck | Y | 73.8 bc |
| | Early Prolific Straightneck | Y | 66.5 c |
| | S13-3850 | S | 61.3 c |
| | S12-3757 | Z | 40.2 d |
| | Black zucchini | Z | 37.3 d |
| | Daytona | Y | 10.3 e |
| | S7-3751 | Z | 8.0 e |
| | S1-3650 | Y | 7.4 e |
| | S3-3652 | Y | 6.6 e |
| | S5-3655 | Y | 5.0 e |
| | S2-3651 | Y | 3.4 e |
| | Cracker | Y | 2.2 e |
| | Crookneck | Y | 2.2 e |
| | S4-3654 | Y | 0.0 e |
| | S6-3656 | Y | 0.0 e |
| | S8-3752 | Y | 0.0 e |
| | Seneca Prolific | Y | 0.0 e |
| | Goldbar | Y | 0.0 e |
| | Sundance | Y | 0.0 e |
| | Butterbar | Y | 0.0 e |
| | Goldneck | Y | 0.0 e |
| | Gold Rush zucchini | Z | 0.0 e |
| | Bennings Greentint | S | 10.2 e |
| | White Bush scallop | S | 9.4 e |
| *C. maxima* | Golden Hubbard | W | 15.7 e |
| *C. ficifolia* | Malabar gourd | W | 3.9 e |
| *C. moschata* | Waltham butternut | W | 3.2 e |
| *C. mixta* | Greenstripped cushaw | W | 1.2 e |
| *C. pepo* var. *pepo* | Connecticut field pumpkin | W | 0.0 e |

[y] Fruit type: WM = watermelon, Y = yellow summer squash, Z = zucchini summer squash, S = scalloped summer squash, W = winter squash, fruit type reflected in cultivar name. Fruit type information on accessions S1-S13 provided by M. Holton, Assistant Plant Breeder, Northrup King Seed Co., Woodland, CA.

[z] Percentage of plants showing symptoms of wilt of severe stunting. Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

## RESULTS

**Host range.** Ten cultivars showed significantly ($P = 0.05$) more wilt than the controls and other cultivars and were considered susceptible to *F. oxysporum* f. sp. *niveum* (Table 1). These included the four yellow straightneck cultivars, Golden Eagle, Hyrific, Straightneck, and Early Prolific Straightneck, and five zucchinis: Black zucchini and four zucchini accessions, S9-3753, S10-3754, S11-3756, and S12-3757. One scallop-fruited accession was also susceptible (S13-3850). Percentage wilt among the susceptible cultivars ranged from 37 to 100%.

Two cultivars, Golden Eagle and Hyrific, and the zucchini accessions S9-3757 and S11-3756 were as susceptible as the watermelon check, exhibiting 89–100% wilt. Black zucchini and zucchini accession S12-3757 have some resistance although they still exhibited a relatively high level of disease (37 and 40% wilt, respectively).

All other cultivars tested had less than 15% wilt and were considered resistant to *F. oxysporum* f. sp. *niveum*. Included among these cultivars are the popular yellow squashes Crookneck and Seneca Prolific, the scallop cultivars White Bush Scallop and Bennings Greentint, and all of the winter squashes tested.

Symptoms of watermelon Fusarium wilt in squash are similar to those in watermelon (Fig. 1A). Yellowing of the leaves occurs within 10–14 days, followed by stunting, wilting of the leaves, and stem collapse. Internal vascular browning was common but did not always occur. External stem discoloration extending from a few millimeters below the soil line to 2–3 cm above the soil line, along with a soft cortical root rot, was common. With the low inoculum levels used in this study, the most frequently observed symptoms were yellowing of the leaves and stunting. Death of susceptible cultivars usually occurred 3–4 wk after planting in infested soil.

**Light microscopy of stem tissue.** Mycelia and vascular occlusions were readily observed throughout the metaxylem in transverse stem sections of inoculated susceptible squash cultivars (Fig. 2A), similar to those observed in infected watermelons. Similar occlusions and mycelia were also observed in the xylem of the inoculated resistant cultivar Crookneck (Fig. 2B) although with much less frequency and in considerably fewer vessels. Rarely were well-defined tyloses observed in either susceptible or resistant cultivars.

**Recovery of the pathogen from inoculated plants.** The pathogen was recovered from stem and root sections of diseased plants. It was culturally indistinguishable from the type culture (ATCC #18467) when compared on Komada medium, PDA, and potato-sucrose agar. It was also indistinguishable microscopically from the type culture, producing typical microconidia and macroconidia on phialides characteristic of *F. oxysporum* (5).

Isolates recovered from Early Prolific Straightneck were used to inoculate the Fusarium wilt-susceptible watermelon Tendergold. The isolate recovered from squash was equally pathogenic to watermelon as was the original type culture, killing 100% of the plants within 2 wk (Fig. 1B).

## DISCUSSION

This study indicates that *F. oxysporum* f. sp. *niveum* can be a severe pathogen on certain summer squash cultivars of the species *Cucurbita pepo* var. *melopepo*. It also confirms previous reports that the winter squashes, specifically cushaw (*Cucurbita mixta*), several pumpkin cultivars (*Cucurbita pepo* var. *pepo*), and Golden Hubbard (*Cucurbita maxima*) are resistant to the watermelon wilt pathogen (4,12,13,19). In addition, representative cultivars of *Cucurbita moschata* (Waltham butternut) and *Cucurbita ficifolia* (Malabar gourd) were also resistant.

Taubenhaus (19) indicated that although both squash and watermelon suffered from Fusarium wilts, the causal agents were distinct from each other. He repeatedly grew watermelons, wild citron, squashes, muskmelons, gourds, and many noncucurbit species on "watermelon-sick soil" and "squash-sick soil." In all cases, only squash died on "squash-sick soil" and only watermelons died on "watermelon-sick soil."

Isolations from diseased plants yielded two apparently distinct *Fusarium* spp.: *F. niveum* from watermelons and *F. cucurbitae* from squash. *F. cucurbitae* was apparently a species novum proposed by Taubenhaus, although he did not mention it as such. Wollenweber and Reinking (22) noted that this species was inadequately described and was not included in their species key. Whether this organism was a forma specialis of *F. oxysporum* or the root rot pathogen *F. solani* f. sp. *cucurbitae* cannot be determined. The root rot pathogen has been observed to cause wilt symptoms on squash similar to wilt of watermelon caused by *F. oxysporum* f. sp. *niveum* (18) and thus could have been the organism described by Taubenhaus. Snyder and Hansen (17), however, do not mention *F. cucurbitae* in their revision of the *Fusarium* section Martiella.

*F. solani* f. sp. *cucurbitae* has been demonstrated as pathogenic to squashes and pumpkins primarily and although watermelons are susceptible to laboratory inoculation, they are seldom infected naturally (20). Therefore, one might expect to find, as Taubenhaus (19) did, that squash wilt *Fusarium* would not cause disease in watermelon.

Taubenhaus (19) also demonstrated that several squash varieties were resistant to the watermelon wilt organism. Some of these (cushaw and pumpkin)

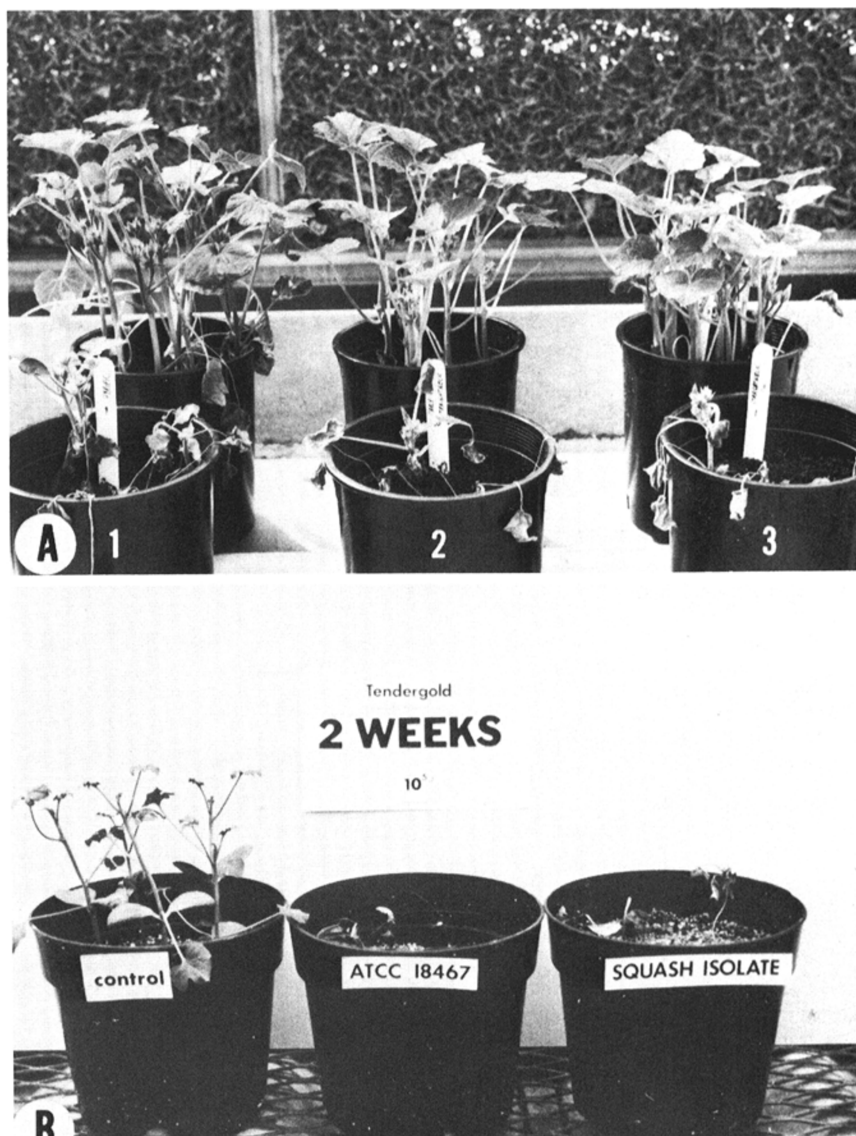


**Fig. 1. (A)** Three summer squash cultivars showing symptoms of Fusarium wilt 4 wk after planting in infested soil. Soil was infested with $6.25 \times 10^5$ spores/L soil prior to planting. Control plants growing in uninfested soil are shown in the background. 1 = Hyrific, 2 = Early Prolific Straightneck, 3 = Straightneck. **(B)** Wilt-susceptible watermelon (Tendergold) seedlings 2 wk after inoculation with *Fusarium oxysporum* f. sp. *niveum* ATCC #18467 or with the *Fusarium* isolate recovered from diseased squash plants. Each inoculum caused 100% wilt in watermelon and they were indistinguishable from each other. Control plants were dipped in diluted sterile culture medium.

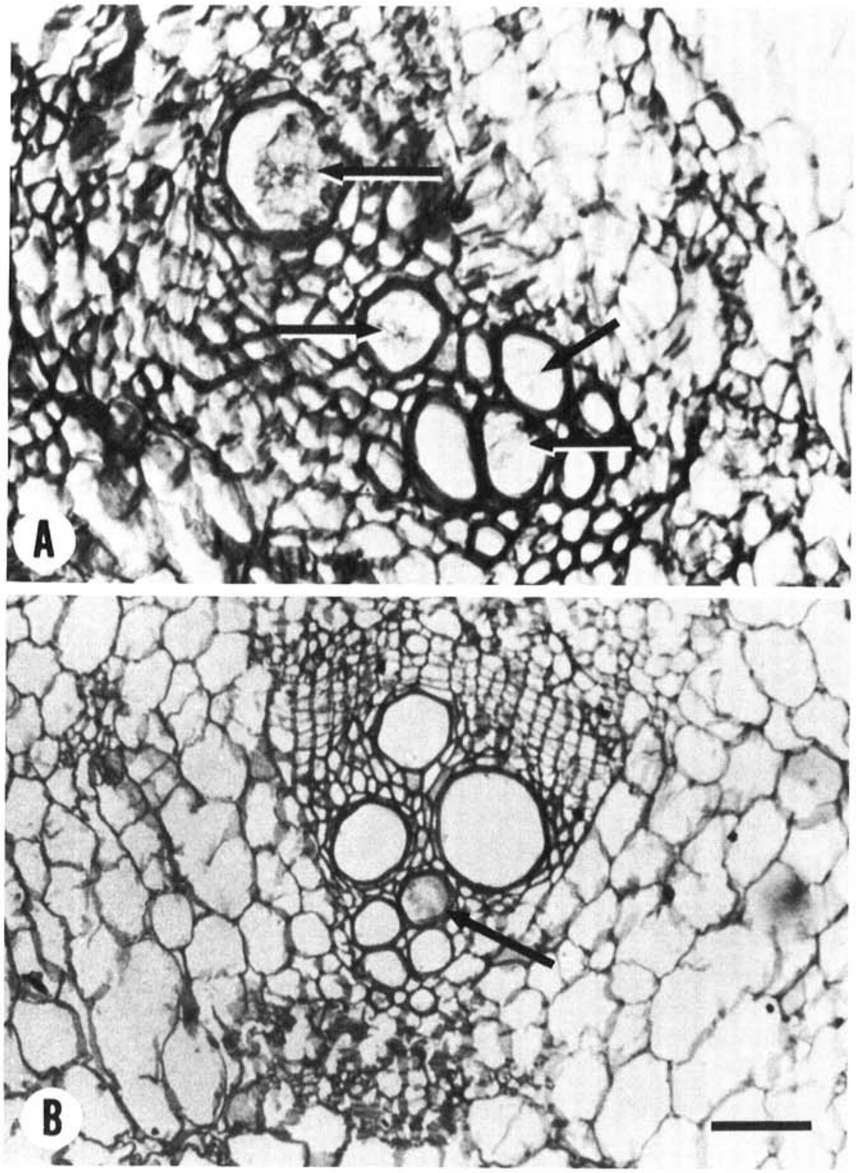


**Fig. 2.** Transverse sections of stem tissue from susceptible and resistant summer squash cultivars. **(A)** Early Prolific Straightneck (susceptible) showing mycelia and vascular occlusions in many metaxylem vessels (arrows). **(B)** Crookneck (resistant) showing vascular occlusions in only one metaxylem element. Other vessels are free of any detectable mycelium. Both sections were made 2 wk after inoculation. Scale bar = 20 $\mu$m.

were tested in this study and were also found resistant. Taubenhaus (19), however, did not identify the other cultivars he tested (referred to as "squash") so it is impossible to determine how inclusive his study was.

The literature before and for a short period after Snyder and Hansen's revision of the section Elegans (16) referred to *Fusarium* spp. that caused wilt of cucurbit species as either *F. niveum* E. F. Sm., *F. bulbigenum* var. *niveum* (E. F. Sm.) Wollenw., or *F. cucurbitae* (11,15,19). Listed hosts of the pathogen included *Cucumis melo* (muskmelon), *Cucumis sativus* (cucumber), and *Cucurbita maxima* and *Cucurbita pepo* (winter and summer squashes). A major plant disease index published in 1926 lists all of the above as suscepts of *F. niveum* (1). These hosts are also listed as suscepts of the watermelon wilt pathogen in two later indices (2,21).

Our findings reveal that *F. oxysporum* f. sp. *niveum* has a wider host range than previously known, and the list of hosts for this pathogen now includes cultivars of summer squash, *Cucurbita pepo* var. *melopepo*. Both resistance and susceptibility can be found in yellow summer squash, zucchini, and scallop-fruit type cultivars. Resistance does not appear to be due to inability of the fungus to penetrate the host tissue but rather to a reduced level of colonization once inside. Similar results have been reported for other Fusarium wilt pathogens (3,6,7,14).

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