Cultivar-Specific Induction of Soil Suppressiveness to Fusarium Wilt of Watermelon

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ABSTRACT

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In a long-term monoculture of watermelon cultivars, most of the cultivars wilted severely after 4–5 yr regardless of previously described levels of resistance to *Fusarium oxysporum* f. sp. *niveum*. Only the resistance in Smokylee and Crimson Sweet was stable in the monoculture, and only Crimson Sweet continued to have acceptable yields throughout the monoculture. Crimson Sweet, only moderately resistant to Fusarium wilt in greenhouse tests, had a unique resistance that was effective throughout the 7-yr monoculture. When soil was collected from the Crimson Sweet plot and assayed, counts of propagules of *F. oxysporum* were not significantly lower than in other cultivar plots, but susceptible cultivars did not wilt when planted in this soil. In soil infested with *F. o. niveum* at 1.5×10^3 conidia per gram, there was 70–100% wilt of Florida Giant, Charleston Gray, or Crimson Sweet in fallow soil or Florida Giant monoculture plot soil; however, there was less than 35% wilt in soil from the Crimson Sweet plots. The suppressive factor(s) in Crimson Sweet soil was sensitive to fumigation with methyl bromide and to moist heat at 70 C for 30 min. The unique resistance of Crimson Sweet to Fusarium wilt in monoculture appears to result from the promotion of a biological control factor in the soil.

Fusarium wilt of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) caused by *Fusarium oxysporum* f. sp. *niveum* (E. F. Sm.) Snyd. & Hans. is a major production problem wherever watermelons are grown. Resistant cultivars and a long rotation are the only controls used by Florida watermelon growers (6). Watermelon growers in Florida are finding it increasingly difficult to find pastureland that has not been used for watermelon culture for 8–10 yr. With the increasing cost of renting pastureland for watermelon production, there is a critical need to shorten the intervals of crop rotation.

Watermelon cultivars are described as resistant or susceptible to Fusarium wilt, but they actually form a continuum from susceptible to highly resistant (6,11). Highly resistant cultivars have not been widely accepted by commercial growers because of less desirable horticultural characteristics (6). Perhaps rotation intervals could be shortened if horticulturally desirable, highly resistant cultivars were available. To evaluate this possibility, we compared watermelon cultivars with a gradation of wilt resistance in a 4-yr monoculture for the development of Fusarium wilt and for the increase in propagules of *F. o. niveum* in the soil (8). As expected, with most cultivars the year-to-year increase of Fusarium wilt was inversely related to the resistance rankings of the cultivars. However, Crimson Sweet, ranked as moderately resistant, was a surprising exception; it had the lowest rate of increase in Fusarium wilt of the 10 cultivars tested.

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The purpose of this study was to evaluate Crimson Sweet and the other cultivars in a prolonged monoculture and to determine why the resistance in Crimson Sweet is uniquely more stable in a monoculture than that of other cultivars. A preliminary report was made on this work (7).

MATERIALS AND METHODS

Monoculture of watermelon cultivars. Ten watermelon cultivars representing the continuum of wilt resistance (6) were grown in a long-term cultivar monoculture as previously described (8). The 10 cultivars, in order from most resistant to most susceptible, were Smokylee, Calhoun Gray, Dixielee, Sugarlee, Crimson Sweet, Charleston Gray, Jubilee, Sugar Baby, Congo, and Florida Giant. Plots were marked by permanent posts so that each cultivar could be monocropped in the same plots throughout the test.

Watermelon seedlings that wilted and died were counted two to three times weekly during the first 5 wk after emergence and total wilt percentages were calculated. At maturity, fruits were harvested and weighed for yield.

Propagule counts of *F. oxysporum* and wilt bioassay. In March or April of each year, soil samples were collected from the field plots for the determination of propagule counts and for greenhouse bioassays for inoculum potential of *F. o. niveum*. Six subsamples were taken from the top 20 cm of soil in each plot and combined to make a 100-g sample. Numbers of propagules of *F. oxysporum* per gram of air-dried soil were determined by plating soil dilutions on Komada's selective medium (9). Five-gram subsamples of soil were added to 45 ml of sterile water and stirred for 10 min. Five milliliters of this solution was added to 95 ml of 0.1% agar in water. While being stirred, 1 ml of this suspension was

pipetted onto each of five plates and spread over the surface by tilting the plates. Colonies of *F. oxysporum* were counted 8–10 days later.

In the 5th and 7th years of the monoculture, the proportions of the propagules of *F. oxysporum* that were *F. o. niveum* were determined by pathogenicity tests. Twenty isolates of *F. oxysporum* were randomly selected from dilution plates of soil from plots of each of the cultivar monocultures. The isolates were grown in liquid broth culture (12). The inoculum was prepared by filtering the cultures through eight layers of cheesecloth and adjusting the concentration of conidia to 5×10^5 per milliliter. Twenty seedlings of Florida Giant watermelon were root-dipped in the inoculum and transplanted into a soil mix. Those isolates that produced wilt in the watermelon in the 4-wk test were considered to be *F. o. niveum*.

Greenhouse bioassays for Fusarium wilt were conducted by planting seeds of Florida Giant in pots containing field soil. Two 10-cm-diameter pots were used for soil from each field plot and 10 seeds were planted per pot. Greenhouse temperatures ranged from about 22 C to 30 C during the bioassays. Wilted seedlings were removed and counted daily for 5 wk after planting, and the percentage wilt was calculated at the end of this time.

Infestation of the soil with *F. oxysporum* f. sp. *niveum*. Isolates of *F. o. niveum* were obtained from stems of naturally infected plants in the field plots. These isolates were highly aggressive, similar to race 2 (12). Inoculum was prepared from liquid broth culture (12). The composition of the medium was K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; yeast extract, 1.0 g; L-asparagine, 2.0 g; glucose, 30.0 g; Fe-EDTA, 0.01 g; and deionized water, 1 L. Liquid cultures were grown for 4–6 days at 25 C. Each culture was filtered through four layers of cheesecloth and the concentration of microconidia then was determined with a hemacytometer and adjusted to 10^6 per milliliter. One milliliter of this suspension was diluted in 40 ml of water and added to the surface of the soil in a 10-cm-diameter pot. This gave approximately 1.5×10^3 conidia of *F. o. niveum* per gram of soil.

Fumigation and heat treatment of soil. Field soil was brought into the greenhouse and fumigated with methyl bromide in a chamber for 24 hr. Heat treatment to selectively eliminate microorganisms from the soil was also utilized on field soil samples (2,3). About 2.5 kg of soil was placed in a glass container in a large covered pot of hot water on a burner. The temperature of the soil was monitored with thermometers. Heat treatments were for 30 min.

Statistical analyses. Analyses of variance were performed for all experiments, and means were compared by Duncan's multiple range test ($P = 0.05$). Percentage wilt data were analyzed after transformation to arc sine $\sqrt{x}$. Logarithmic transformations were used on populations of *F. oxysporum* prior to analysis.

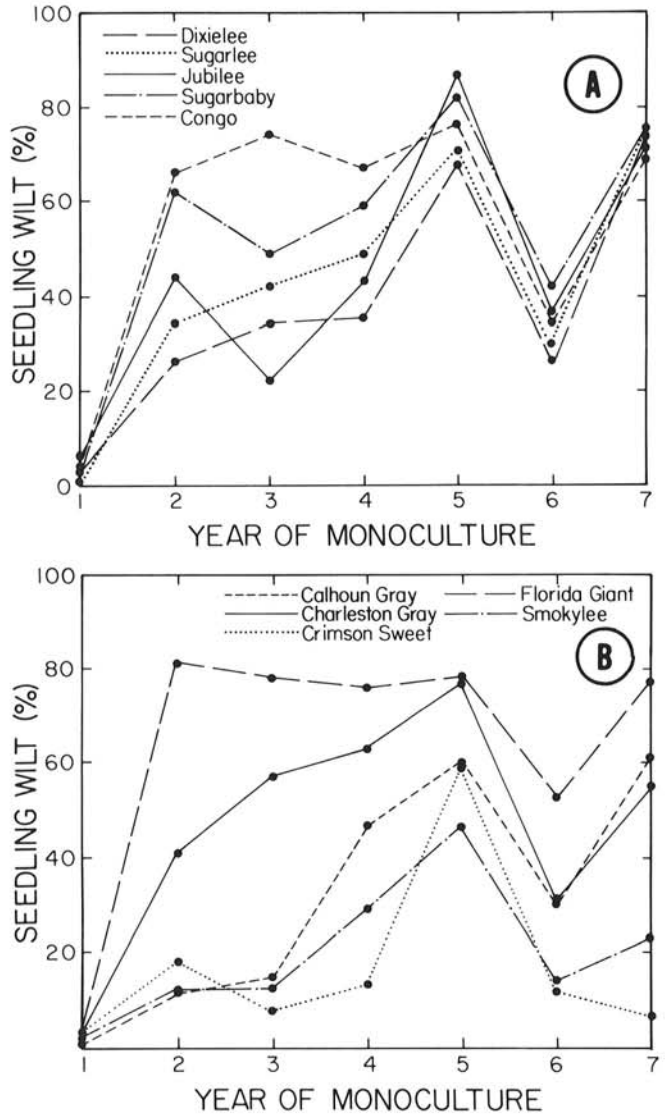

Fig. 1. Yearly development of seedling wilt caused by *Fusarium oxysporum* f. sp. *niveum* in 10 watermelon cultivars, each grown in long-term monoculture. **A,** Cultivars Dixielee, Sugarlee, Jubilee, Sugar Baby, and Congo; **B,** cultivars Calhoun Gray, Charleston Gray, Crimson Sweet, Florida Giant, and Smokylee.

RESULTS

Field monoculture of watermelon cultivars. As previously reported (8), the rate of increase in Fusarium wilt during the first 4 yr of a watermelon cultivar monoculture was inversely related to the published resistance rankings of these cultivars, except for Crimson Sweet. However, by the 5th, 6th, and 7th years of the monoculture, many cultivars wilted to a similar level (Fig. 1A). There was as much seedling wilt in the resistant Dixielee and Sugarlee as in the susceptible Congo and Sugar Baby, and by the 6th year of the monoculture the highly resistant Calhoun Gray was as severely affected by wilt as was Charleston Gray (Fig. 1B). Smokylee (highly resistant) and Crimson Sweet (moderately resistant) continued to have significantly less wilt than all other cultivars, except in the 5th year ($P = 0.05$). There is no apparent explanation for the higher wilt incidence in these two cultivars in the 5th year. There was a similar year-to-year variation in level of wilt in all cultivars, which probably results from environmental conditions.

Only four cultivars still produced harvestable fruit by the 6th and 7th years of monoculture (Fig. 2). Of these four, Crimson

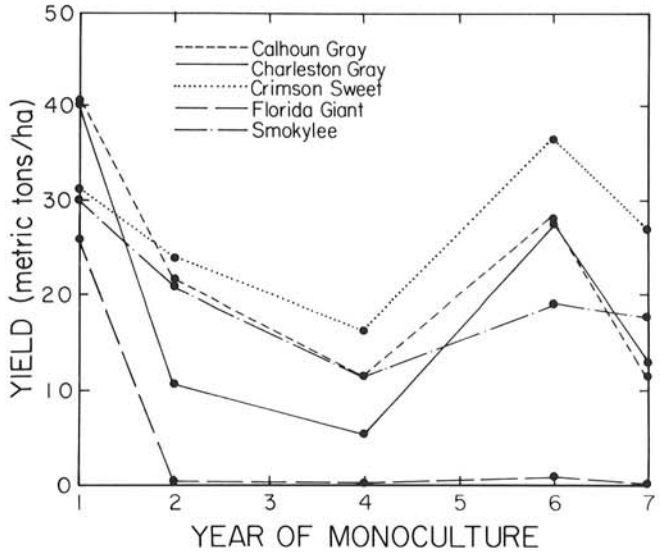

Fig. 2. Annual yields of five watermelon cultivars grown in long-term monoculture.

Sweet had significantly higher yields than the others ($P = 0.05$). The yield of Crimson Sweet was actually higher in the 6th year of monoculture than in the 1st year.

Assays for *F. oxysporum* in the soil. Because both soil populations of *F. o. niveum* and cultivar resistance affected the amount of Fusarium wilt in the field, soil from the monoculture plots was brought into the laboratory for propagule counts of *F. oxysporum* and for a bioassay using a single susceptible watermelon cultivar. Significantly less wilt occurred in the highly susceptible cultivar Florida Giant grown in the soil from plots cropped to Crimson Sweet for 5, 6, and 7 yr than in soil from any other monoculture plot ($P = 0.05$) (Fig. 3). The difference was especially large in the 7th year, with only 5% wilt in Florida Giant grown in soil from the Crimson Sweet plots and with 35% wilt in plants grown in soil from the Charleston Gray plots, the next lowest of the test.

Dilution plating indicated that there were fewer inocula of *F. oxysporum* in the soil from the Crimson Sweet plots (Fig. 4), but the differences were small and not statistically significant ($P = 0.05$). With all cultivars, populations of *F. oxysporum* seemed to have stabilized by the 6th and 7th years of the monoculture. Pathogenicity tests showed that 30–50% of the population of *F. oxysporum* was *F. o. niveum* in the 5th year of the monoculture and 45–55% was *F. o. niveum* in the 7th year. The proportion of *F. o. niveum* present in the soil was not significantly affected by the cultivar that was grown in the plots ($P = 0.05$).

Infestation of soil with *F. oxysporum* f. sp. niveum. In a greenhouse study, fumigated and nonfumigated soil that had never been used for watermelon production and soil from the Crimson Sweet and Florida Giant monoculture plots were infested with *F. o. niveum*. Between 70 and 100% of the plants, whether Florida Giant, Charleston Gray, or Crimson Sweet, developed wilt when grown in the fumigated and nonfumigated fallow soil (Table 1). The more resistant Calhoun Gray had about 50% wilt. In contrast, soil from the Crimson Sweet monoculture plots was suppressive to Fusarium wilt, with only 18% wilted plants in Calhoun Gray and 32–35% in the other three cultivars. Soil from the Florida Giant monoculture plot behaved similarly to fallow soil with all cultivars except Crimson Sweet, where it suppressed wilt.

Fumigation of soil from monoculture plots. Fumigation with methyl bromide eliminated the wilt suppressiveness of Crimson Sweet monoculture plot soil when assessed on Florida Giant after infestation with *F. o. niveum* (Fig. 5). There were more wilted plants in all of the fumigated soils, but the differences between fumigated and nonfumigated treatments were significant only with soil from the Crimson Sweet and Charleston Gray plots ($P = 0.05$). However, there were more wilted plants of Florida Giant grown in the nonfumigated soil from Charleston Gray plots than in soil from the Crimson Sweet plots, indicating that the Charleston Gray soil was not as strongly suppressive as Crimson Sweet soil.

Selective heat treatment of Crimson Sweet monoculture soil. Moist heat applied for 30 min at 70 C to soil from the Crimson Sweet monoculture plot apparently eliminated the suppressive factor (Fig. 6). Florida Giant seedlings in soil treated at 60 C and then infested with *F. o. niveum* had 34% wilt, whereas those in the same soil treated at 70 C prior to infestation had 88% wilt.

DISCUSSION

Resistance of watermelon cultivars to Fusarium wilt has usually been determined either by infestation of sterile soil in the greenhouse or by field tests in naturally infested soil where watermelons had recently been grown (6,11). During the first 4 yr of this cultivar monoculture study, the development of Fusarium wilt in the various cultivars agreed with the earlier rankings of wilt resistance (8). However, Crimson Sweet was much more resistant to *F. o. niveum* in the monoculture than it was in greenhouse tests in sterile soil or in naturally infested field soil.

By the 5th year of the monoculture, wilt incidence of most cultivars had reached the same level and stabilized, regardless of wilt resistance ranking from earlier studies (6,11). Apparently it took 4–5 yr for *F. o. niveum* to overcome, in some manner, the resistance of Dixielee, Calhoun Gray, and Sugarlee. Only the

TABLE 1. Effect of soil source on wilt development in watermelon caused by *Fusarium oxysporum* f. sp. *niveum* after infestation of soil in greenhouse

Soil source[x]	Wilt per cultivar (%)[y,z]			
	FG	CG	CS	Cal. G
Nonfumigated fallow soil	82 a	72 a	92 a	56 a
Fumigated fallow soil	87 a	79 a	100 a	44 a
Florida Giant plot	79 a	80 a	36 b	44 a
Crimson Sweet plot	33 b	35 b	32 b	18 b

[x] Fallow soil was collected from area that had not previously been planted in watermelon. All four soils were infested with *F. oxysporum* f. sp. *niveum* at 1.5×10^3 conidia per gram.

[y] FG = Florida Giant, CG = Charleston Gray, CS = Crimson Sweet, and Cal. G = Calhoun Gray.

[z] Means in columns followed by same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test; percentage wilt data were analyzed after transformation to arc sine $\sqrt{x}$.

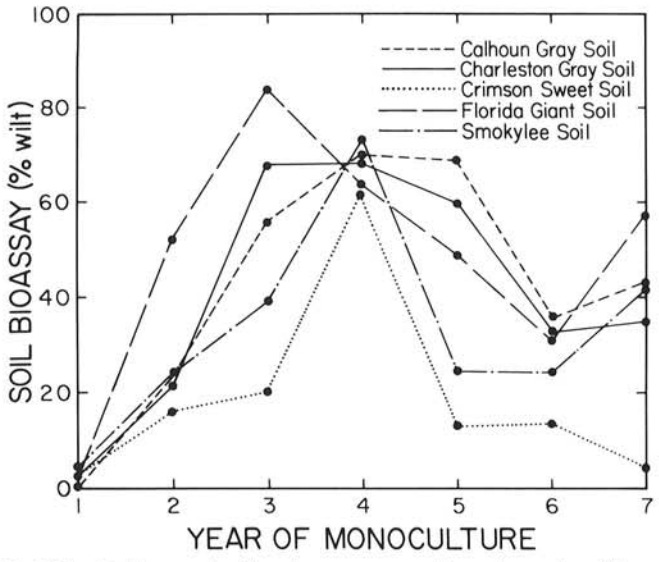

Fig. 3. Yearly bioassay for *Fusarium oxysporum* f. sp. *niveum* in soil from plots planted every year to same indicated cultivar. Susceptible cultivar Florida Giant was used in bioassay.

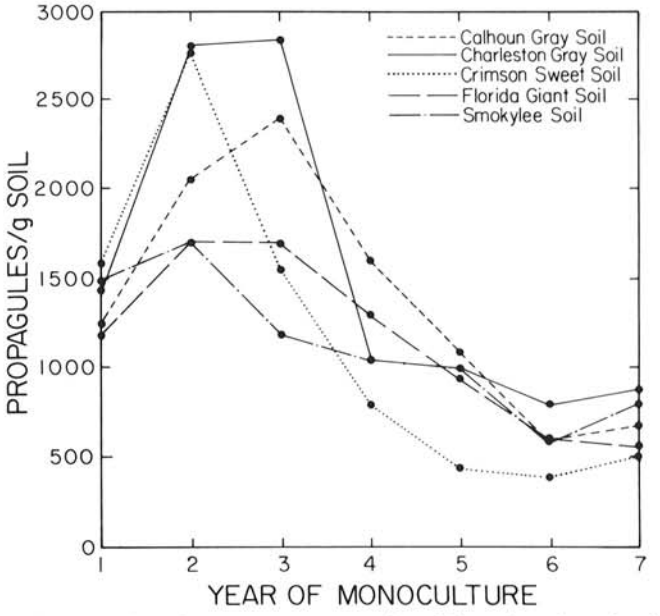

Fig. 4. Population of *Fusarium oxysporum* in soil from plots planted each year to same indicated cultivar.

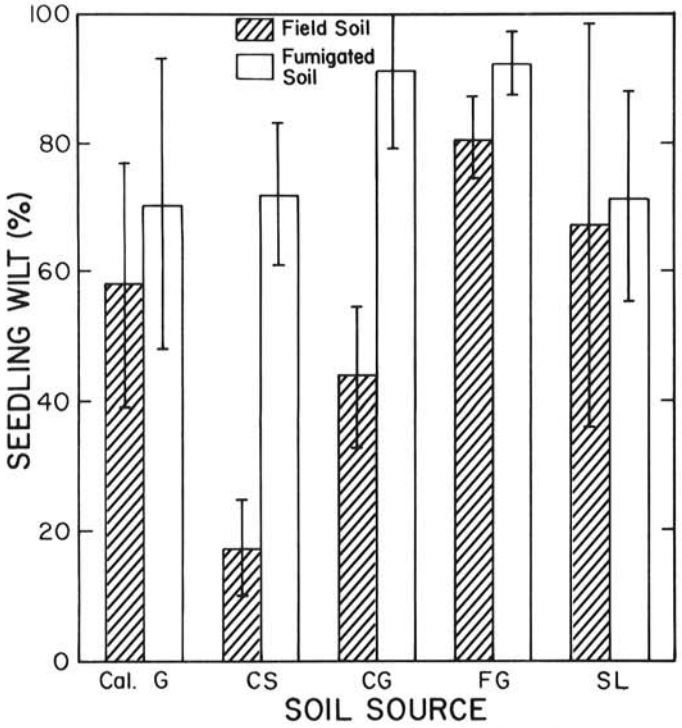

Fig. 5. Development of seedling wilt in fumigated and nonfumigated soil from plots with indicated cultivar grown in monoculture. Each soil was infested with conidia of *Fusarium oxysporum* f. sp. *niveum* at 1.5×10^3 per gram of soil and planted with susceptible cultivar Florida Giant. Mean values ± SE are results of four replicates. Cal. G = Calhoun Gray, CS = Crimson Sweet, CG = Charleston Gray, FG = Florida Giant, and SL = Smokylee.

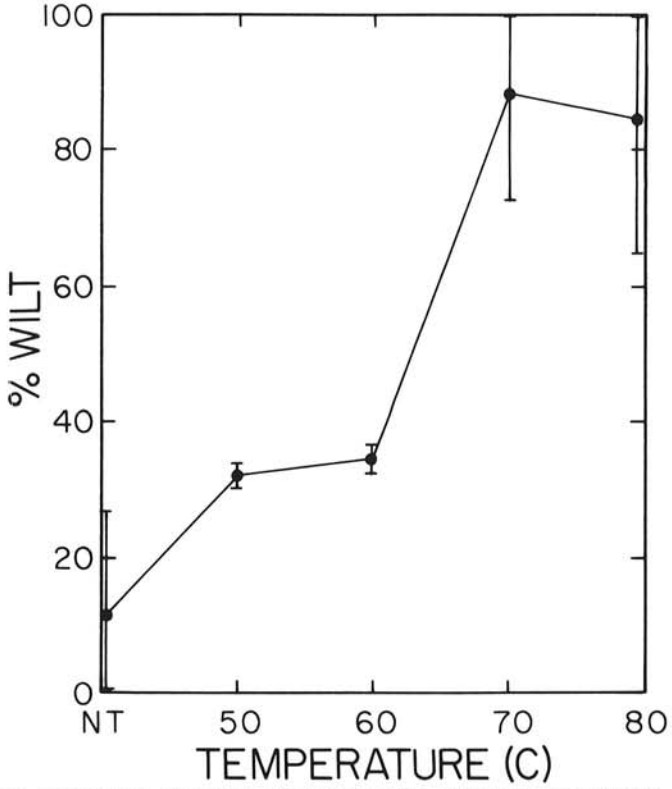

Fig. 6. Incidence of seedling wilt in susceptible cultivar Florida Giant in heat-treated soil from Crimson Sweet monoculture plot. Soil was subjected to moist heat treatments at indicated temperatures for 30 min and then reinfested with *Fusarium oxysporum* f. sp. *niveum* at 1.5×10^3 conidia per gram of soil. Vertical bars indicate ± SE.

resistance of Crimson Sweet and Smokylee was expressed through the 7-yr monoculture. The loss of expressed resistance in Calhoun Gray, Dixielee, and Sugarlee was not related to high populations of *F. o. niveum*, since propagule counts in the test plot soils were similar for resistant and susceptible cultivars. Perhaps highly virulent strains of *F. o. niveum*, or strains that are not controlled by the resistance genes of these cultivars, could have developed after 4–5 yr of monoculture. This loss of resistance is currently being studied.

Of interest was the stable resistance in the monoculture of Crimson Sweet and Smokylee. The performance of Smokylee could have been the result of its high-type genetic resistance to Fusarium wilt, which can be demonstrated in greenhouse tests. Crimson Sweet does not have a high level of resistance in greenhouse tests in sterile, infested soil. Although propagule counts of *F. oxysporum* were low in soil where Crimson Sweet had been grown in monoculture, they were not significantly lower than those in other plots and would not appear to explain totally the performance of Crimson Sweet in a monoculture. Rather, the resistance of Crimson Sweet in a monoculture appears to be the result of the promotion of suppressiveness to *F. o. niveum* in the soil. The suppressive factor(s) was sensitive to fumigation with methyl bromide and to moist heat for 30 min at 70 C. Only Crimson Sweet promoted the development of this suppressive factor to a level that allowed the production of acceptable yields of watermelon in a long-term monoculture; however, there was some suppression in the Charleston Gray soil.

This is apparently the first report of a cultivar-specific induction of suppressive soils for a disease. With take-all of wheat (*Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker), take-all decline (15) is thought to be a specific suppression resulting from a qualitative change in the soil microbial population following monoculture of wheat (5), but it is not cultivar specific. Soils that are suppressive to *F. oxysporum* are known to occur in many places around the world (10,13,16). However, these suppressive soils are usually of a particular type and are not necessarily induced by a particular host. Nonpathogenic strains of *F. oxysporum* are abundant in some of these suppressive soils and have been implicated as the suppressive factor (1,14). However, the suppressive soil from the Crimson Sweet plots did not have significantly higher populations of nonpathogenic *F. oxysporum* than the populations found in soil from other plots. It is possible that Crimson Sweet grown in a monoculture enriched a particular nonpathogenic *F. oxysporum* or weakly pathogenic *F. o. niveum* that could be the suppressive factor.

Most of the emphasis in biological control systems has been directed at selecting the antagonist. Future research directed at development of cultivars more suitable for antagonist population increase also may prove fruitful (4). The Crimson Sweet watermelon cultivar appears to offer a unique opportunity to study host-mediated genetic control of naturally occurring antagonists to a specific disease. From a practical standpoint, combining the "direct" resistance—that resistance expressed in greenhouse infestations of sterile soil—of Calhoun Gray or Smokylee with the "indirect" resistance—promotion of suppressive factor(s) in the soil—of Crimson Sweet might produce a cultivar that can be grown commercially in a short rotation or monoculture in Florida.

Although Crimson Sweet was uniquely able to promote the buildup of the suppressive factor(s), the suppressiveness that developed after the Crimson Sweet monoculture was effective on all cultivars. When this antagonist(s) is identified, perhaps it can be used directly as a biological control for Fusarium wilt of all watermelon cultivars.

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