Evaluation of Virus Resistant Squash and Interaction with Reflective and Nonreflective Mulches

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SUMMARY. A 3-year study to evaluate mulch type (reflective and black) and new virus resistant summer squash (*Cucurbita pepo* L.) varieties was undertaken. In the first year of the study (1996), in Shorter, Ala., under slight virus pressure, silver painted mulch suppressed virus symptoms through the final evaluation 2 months after planting. In addition, virus symptoms were significantly more prevalent on 'Dixie' compared to 'Supersett', 'Tigress', 'HMX 5727', 'Jaguar', 'Destiny III', and 'Prelude II'. In the second year (1997), two different experiments were conducted in Savannah, Ga., where there was no virus pressure. In the first experiment at the Savannah location, 'Tigress' and 'HMX 6704' had significantly higher yields than 'Destiny III', 'Prelude II', 'Puma', 'Jaguar', 'Meigs', 'Dixie', and 'Supersett'. In the second Savannah experiment, 'Prelude II' and 'Destiny III' had significantly higher yields than 'Zucchini Elite', 'Supersett', 'HMX 6704', and 'Jaguar'. In 1998 at Shorter, there was no difference in virus incidence based on mulch used. Although there were differences in virus incidence among varieties, the lowest incidence was 70% of plants infected for 'Prelude II'. In addition to field evaluations, these varieties were evaluated for resistance to zucchini yellow mosaic virus under greenhouse conditions. Varieties HMX 7710, HMX 6704, Puma, Tigress, Prelude II, Jaguar, and Destiny III were significantly more resistant compared to varieties Zucchini Elite, Meigs, Supersett, and Dixie. In conclusion, reflective mulch was effective only under slight virus pressure.

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Summer squash (*Cucurbita pepo*) is an important vegetable crop in many parts of the world and is particularly important in the southeastern United States. Production, however, in the southeastern U.S. is generally limited to spring and early summer. Summer and fall production is greatly curtailed due to aphid transmitted viruses. This is particularly true for yellow summer squash because of the green mottled color of virus infected fruit which renders them unsaleable.

Several different methods of controlling or reducing the severity of virus infection in squash have been used. The most important of these is time of planting. In Georgia, for example, spring production of zucchini and yellow summer squash is 7,360 acres (2,978 ha) versus 3,824 acres (1,548 ha) of fall production even though fall prices are much higher (Mizelle, 1999).

Stylet oils have also been shown to be effective in reducing virus incidence. In one such trial a proprietary oil was shown to reduce the incidence of watermelon mosaic virus (WMV, formerly WMV II) in grezoni squash (*C. pepo* L.) (Mansour and Al-Musa, 1982). These oils have also been shown to be particularly effective, when used in combination with reflective mulch (Mansour, 1997).

Use of reflective mulch has been shown to reduce or delay the onset of virus incidence in squash (Brown et al., 1993). This has been known certainly since the mid-1960s (Smith et al., 1964). Reflective mulches are effective in helping control virus diseases. However, they do not eliminate virus infection for the entire season, but rather delay the onset of virus symptoms.

More recently, several new varieties with reported resistance to several different virus diseases have been introduced. Some of these yellow summer squash varieties do not have true resistance, but do have the precocious yellow gene, which results in a lack of green pigmentation in the fruit peduncle. Subsequently, even when virus infects these varieties, fruit do not show the green mottling and thus remain salable.

Another source of resistance is transgenic varieties. Recently the protocols have been worked out to transfer genetic material from one species to another. This has been accomplished with several different cucurbit species (Chee and Slightom, 1991; Clough and Hamm, 1995; Gonsalves et al., 1994). In addition, it has been known for several years that plants infected with a mild strain of a virus will induce plant resistance to more virulent strains, referred to as cross protection. Cross protection has been exploited by incorporating the coat protein gene from the target virus into the host plant's genome (Clough and Hamm, 1995). This renders the variety resistant to that specific virus. Several varieties have been introduced with this type of resistance. Two such varieties are 'Prelude II' and 'Destiny III'. 'Prelude II' has resistance to zucchini yellow mosaic virus (ZYMV) and WMV. 'Destiny III' has resistance to ZYMV, WMV, and cucumber mosaic virus (CMV) (J. White, personal communication).

In addition to transgenic resistance, varieties have been developed with virus resistance from interspecific crosses of *C. pepo* and *C. moschata* (Duchesce ex Lam.) Duchesce ex Poir., (Superak et al., 1993). Several varieties with resistance to ZYMV have been introduced from this source including, 'Jaguar', 'Tigress', and 'Puma'.

Squash varieties, like most vegetables, are routinely evaluated for performance. Recent introductions of new virus resistant varieties have been evaluated at several locations including, Mississippi, Alabama, and Kentucky (Cushman and Horgan, 1998; Rowell et al., 1999; Simonne et al., 1998).

The purpose of this study was to

evaluate virus incidence and yield in new virus resistant squash varieties with and without reflective mulch and their interaction.

Materials and methods

There were four field experiments conducted during this study. The experiments were a factorial design with variety and mulch type as the factors. The experiments were arranged in a randomized complete block design (RCBD) of three replications in Shorter, Ala., and four replications in Savannah, Ga.

Experiments (Expts. 1 and 4) were conducted in 1996 and 1998 at the E.V. Smith Research Center, Shorter, on a Norfolk-Orangeburg loamy sand association (fine, loamy, siliceous, thermic Typic Kandiudults). Fertilizer was applied preplant according to soil test recommendations for Alabama (Adams et al., 1994). In total, 462 lb / acre (518 kg·ha^{-1}) of 13N–5.7P–10.8K fertilizer was incorporated before planting. In addition, 10 lb / acre (11.2 kg·ha^{-1}) of $CaNO_3$ was delivered through the drip irrigation system starting in the second week after transplanting and continuing for 4 weeks. The soil was prepared with agriculture plastic laying equipment, which pressed a 4-inch (10 cm) high × 2.5-ft (0.76 m) wide bed covered with 1.25-mil [0.00125 inch (0.032-mm)] black polyethylene mulch (Edison Plastics, Co., Washington, Ga.) Beds were 6 ft (1.8 m) from row-center to row-center. A single trickle irrigation line (T-Tape, T Systems International, Inc., San Diego, Calif.) was placed slightly off-center on each bed directly under the black plastic mulch. Soil was irrigated to field capacity when soil was below 50% of field capacity as determined by feel.

In 1997, experiments (Expts. 2 and 3) were conducted at the Bamboo Farm and Coastal Garden, Savannah, on an Ocilla-Pelham-Albany association (loamy fine sand). Beds were prepared with agriculture plastic laying equipment, which prepared a pressed 11-inch (28-cm) high × 2.5-ft wide beds covered with 1.25-mil black polyethylene mulch (Edison Plastics, Co.). Beds were 6 ft (1.8 m) from row-center to row-center with a single trickle irrigation line (T-Tape, T Systems International, Inc.) placed slightly off-center on each bed directly under the black plastic mulch. Fertilizer was supplied through the trickle irrigation tape at a rate of 6.5 lb / acre (7.3 kg·ha^{-1}) of KNO_3, 17.7 lb / acre (19.8 kg·ha^{-1}) of $CaNO_3$, and 10.1 lb / acre (11.3 kg·ha^{-1}) 9N–19.6P–12.4K weekly. Water was added to the soil through a trickle irrigation system when field capacity went below 50% as determined by feel.

The first experiment in Shorter (Expt. 1) included varieties Supersett, Prelude II, Destiny III, HMX 5727, Jaguar, Dixie, and Tigress. Mulch type consisted of black plastic mulch as described above and reflective mulch which was prepared by spraying plots of black plastic with a backpack sprayer with liquid aluminum 920 paint (St. Louis Paint Manufacturing Co., St. Louis, Mo.) diluted to two parts paint to one part paint thinner.

Following bed preparation, 2-week-old plants were transplanted by hand on 23 Aug. 1996. Each plot consisted of 10 plants set 2 ft (61 cm) apart in the row. Harvesting began on 16 Sept. 1996 and continued through 24 Oct. 1996. Fruit were harvested every 2 to 3 d during this period, as they became ready.

The second experiment (Expt. 2) was conducted in Savannah and included varieties Tigress, HMX 6704, Zucchini Elite, Destiny III, Prelude II, Puma, Jaguar, Meigs, Dixie, and Supersett. Mulch type consisted of black plastic mulch as described above and reflective mulch which was prepared by spraying plots of black plastic with a backpack sprayer filled with fibered asphalt aluminum roof coating (Gibson-Homans Co., Twinsburg, Ohio) diluted two parts roof coating to one part paint thinner. Following bed preparation, 2-week-old plants were transplanted by hand on 28 May 1997. There were 10 plants per plot spaced 2 ft apart in the row. Harvest began on 1 July 1997 and continued every few days through 29 July 1997.

The third experiment (Expt. 3) also conducted in Savannah and had the same varieties as Expt. 2. Mulch type consisted of black plastic mulch as described above and reflective mulch plots prepared by hand covering the black plastic with reflective mulch (Colorup, Specialty Ag Equip., Inc., Reedley, Calif.) for those plots requiring the silver mulch treatment. Two-week-old plants were transplanted on 10 Sept. 1997 into the prepared beds. Each plot consisted of 10 plants spaced 2 ft apart in the row. Fruit were harvested from 8 Oct. 1997 to 3 Nov. 1997.

The fourth experiment in Shorter (Expt. 4) repeated Expt. 3. Two-week-old plants were transplanted onto the beds on 7 Aug. 1998. Fruit was harvested beginning on 26 Aug 1998 and continuing every few days through 30 Sept. 1998.

Fungicide applications (triadimefon) to control powdery mildew [*Sphaerotheca fuliginea* (Schl.) Salmon] and / or *Erysiphe cichoracearum* De Candolle were applied as needed. In addition, plants in Expt. 3 were sprayed weekly alternating carbaryl and *Bacillus thurgenensis* (Berliner) insecticides.

One to two weeks after establishing each experiment, plants were evaluated weekly for signs of viral infection, continuing throughout the production cycle. In addition, Expt. 4 had two recently mature leaves inspected weekly for aphids and the number of aphids present counted. Aphids were not identified as to species, and virus presence was not determined.

A greenhouse experiment (Expt. 5) was conducted to determine the response of squash varieties to infection with ZYMV Florida strain. A RCBD was used with three replications. Each entry within a replication had eight seed planted into 11 × 22-inch (28 × 56-cm) flats of peat based media. The first true leaves of each plant were dusted with carborundum. Inoculum was prepared by taking ZYMV-FL infected squash leaves and macerating in a 0.05 M KPO_4 buffer at a 1:2 ratio (wt / vol). This inoculum was then rubbed on the carborundum dusted leaves. This procedure was repeated 1 week later to insure infection. Plants were evaluated 5 weeks after final inoculation on a 0 to 5 scale with 0 = no visible signs of infection and 5 = severe infection symptoms.

Results

Experiment 1. In Expt. 1, 'Supersett' and 'Prelude II' had significantly higher yields than 'HMX 5727', 'Jaguar', 'Dixie', or 'Tigress' with yields of 9,187 and 8,505 lb / acre, (10,299 kg·ha^{-1} and 9,534 kg·ha^{-1}) respectively (Table 1). 'Destiny III' did not differ from 'Supersett' or 'Prelude II'. Mulch surface, whether black or reflective, had no effect on yield in this experiment. In addition, there was no variety by mulch interaction.

Table 1. Yields of summer squash varieties by variety and mulch type at E.V. Smith Research Center, Shorter, Ala., 1996 (Expt. 1).

Variety	Yield[z] (lb/acre)	Yield (kg·ha^{-1})	Fruit type	Virus resistance source
Supersett	9,187 a	10,299	Yellow crookneck	Precocious yellow gene
Prelude II	8,505 a	9,534	Yellow crookneck	Transgenic
Destiny III	8,134 ab	9,118	Yellow crookneck	Transgenic
HMX 5727	5,333 b	5,978	Zucchini	Interspecific
Jaguar	4,844 b	5,430	Zucchini	Interspecific
Dixie	4,010 b	4,495	Yellow crookneck	None
Tigress	2,670 b	2,993	Zucchini	Interspecific
Mulch				
Silvered	6,669	7,476		
Black	5,526	6,195		
Probabilities				
Variety	0.008			
Mulch	0.250			
Variety × mulch	0.208			

[z]Means followed by the same letter within the column are not different by Fisher's Protected LSD ($P \leq 0.05$).

Virus incidence differed by both variety and mulch surface when evaluated on 21 Oct. 1996 (Figs. 1 and 2). 'Dixie' had significantly higher virus infection compared to the other varieties with an average infection rate of 13.9% compared to less than 5% for all other entries. Plants on reflective mulch had a significantly lower virus incidence with an average infection rate of 1.1% compared to 5.2% for black plastic mulch.

EXPERIMENT 2. 'Tigress' and 'HMX 6704' had significantly higher yields compared to 'Destiny III', 'Prelude II', 'Puma', 'Jaguar', 'Meigs', 'Dixie', and 'Supersett' (Table 2). Mulch surface did not affect yield, but the interaction between variety and mulch was significant. There was no virus infection throughout this experiment.

EXPERIMENT 3. 'Prelude II' and 'Destiny III' had significantly higher yields compared to 'Zucchini Elite', 'Supersett', 'HMX 6704', and 'Jaguar' (Table 3). Mulch surface had no affect on yield. In addition, there was no variety by mulch interaction. As in Expt. 2 there was no virus incidence in this experiment.

EXPERIMENT 4. 'Tigress' had significantly higher yields compared to 'Zucchini Elite', 'Meigs', 'Jaguar', 'HMX 6704', 'Destiny III', 'Prelude II', 'Supersett', 'Dixie', and 'Puma' (Table 4). In addition, 'Puma', 'Dixie', 'Supersett', 'Prelude II', and 'Destiny III' had significantly lower yields than 'Tigress' and 'Zucchini Elite'. In addition to differences among varieties,

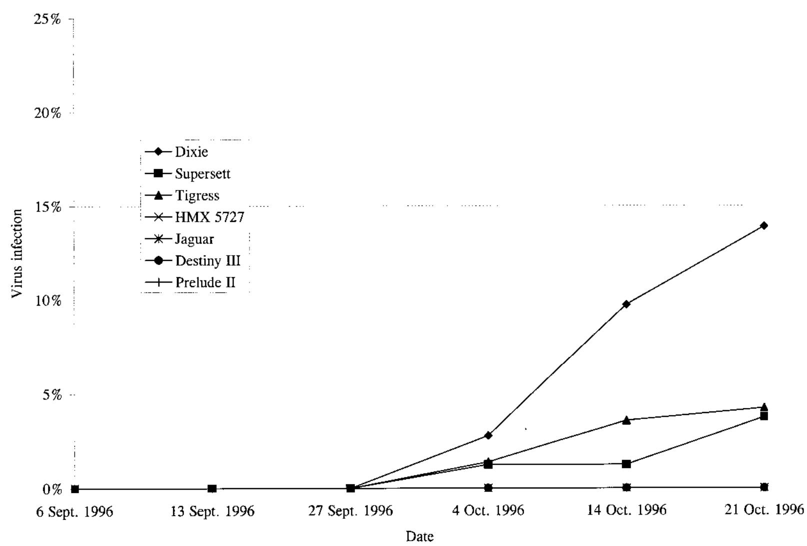

Fig. 1. Percentage virus infection over time by variety in Shorter, Ala. (Expt. 1).

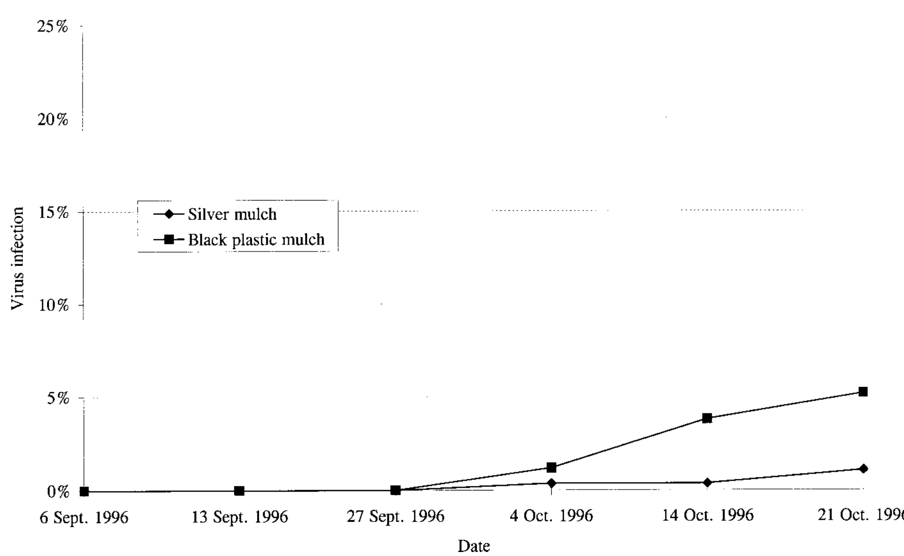

Fig. 2. Percentage virus infection over time by mulch type in Shorter, Ala. (Expt. 1).

Table 2. Yields of summer squash varieties by variety and mulch type at the Coastal Garden and Bamboo Farm, Savannah, Ga., 1997 (Expt. 2). Transplanted 28 May 1997, harvested 1–7 July 1997.

Variety	Yield[z] (lb/acre)	Yield ($kg \cdot ha^{-1}$)	Fruit type	Virus resistance source
Tigress	17,263 a	19,352	Zucchini	Interspecific
HMX 6704	15,321 a	17,175	Zucchini	Interspecific
Zucchini Elite	13,017 ab	14,592	Zucchini	None
Destiny III	11,872 bc	13,309	Yellow crookneck	Transgenic
Prelude II	11,349 bcd	12,722	Yellow crookneck	Transgenic
Puma	11,177 cd	12,529	Zucchini	Interspecific
Jaguar	10,151 cde	11,379	Zucchini	Interspecific
Meigs	9,998 cde	11,208	Yellow crookneck	Precocious Yellow
Dixie	8,114 de	9,096	Yellow crookneck	None
Supersett	7,922 e	8,881	Yellow crookneck	Precocious Yellow
Mulch				
Silvered	11,748	13,170		
Black	11,489	12,879		
Probabilities				
Variety	0.000			
Mulch	0.723			
Variety × mulch	0.036			

[z]Means followed by the same letter within the column are not different by Fisher's Protected LSD ($P \leq 0.05$).

Table 3. Yields of summer squash varieties by variety and mulch type at the Coastal Garden and Bamboo Farm, Savannah, Ga., 1997 (Expt. 3). Transplanted 10 Sept. 1997, harvested 8 Oct. to 3 Nov. 1997

Variety	Yield[z] (lb/acre)	Yield ($kg \cdot ha^{-1}$)	Fruit type	Virus resistance source
Prelude II	7,606 a	8,526	Yellow crookneck	Transgenic
Destiny III	6,131 ab	6,873	Yellow crookneck	Transgenic
Dixie	5,572 bc	6,246	Yellow crookneck	None
Meigs	5,440 bc	6,098	Yellow crookneck	Yellow precocious gene
Tigress	5,019 bc	5,626	Zucchini	Interspecific
Puma	4,451 bcd	4,990	Zucchini	Interspecific
Zucchini Elite	4,434 cd	4,971	Zucchini	None
Supersett	3,970 cd	4,450	Zucchini	Yellow precocious gene
HMX 6704	3,274 d	3,670	Zucchini	Interspecific
Jaguar	1,067 e	1,196	Zucchini	Interspecific
Much				
Silver	4,528	5,076		
Black	4,864	5,452		
Probabilities				
Variety	0.000			
Mulch	0.375			
Variety × mulch	0.270			

[z]Means followed by the same letter within the column are not different by Fisher's Protected LSD ($P \leq 0.05$).

mulch type had an effect on yield. Reflective mulch had greater yields than did black plastic mulch with 10,633 lb / acre (11,920 $kg \cdot ha^{-1}$) compared to 8,391 lb / acre (9,406 $kg \cdot ha^{-1}$). There was no variety by mulch interaction.

There were significant differences among varieties for percent virus infection on the final date of evaluation (Fig. 3, Table 5). 'Prelude II' had significantly lower virus incidence at 70% compared to 'Dixie', 'Meigs', 'Supersett', 'Destiny III', 'Tigress', and 'Jaguar' (Table 5). 'Prelude II' did not differ from 'HMX 6704', 'Puma', and 'Zucchini Elite'. There were no difference based on mulch surface (Fig. 4) and there was no variety by mulch interaction.

Aphid counts were significantly less on 19 Aug., 26 Aug., and 23 Sept. 1998 on reflective mulch compared to black plastic mulch (Figs. 5–6). Aphid counts did not differ among varieties or mulch type on the final day of evaluation 5 Oct. 1998. There was a spike in aphids recorded on 16 Sept. 1998, but there was no difference based on mulch type or variety. In addition, there was no variety by mulch interaction.

EXPERIMENT 5. In the greenhouse evaluation of resistance to ZYMV-FL, 'Zucchini Elite', 'Supersett', 'Meigs', and 'Dixie' had significantly more se-

Table 4. Yield and description of summer squash varieties at E.V. Smith Research Center, Shorter, Ala., 1998 (Expt. 4).

Variety	Yield[z] (lb/acre)	Yield (kg·ha^{-1})	Fruit type	Virus resistance source
Tigress	15,074 a	16,898	Zucchini	Interspecific
Zucchini Elite	11,650 b	13,060	Zucchini	Susceptible
Meigs	10,115 bc	11,339	Yellow Summer	Precocious Yellow
Jaguar	9,632 bc	10,797	Zucchini	Interspecific
HMX 6704	9,512 bc	10,663	Zucchini	Interspecific
Destiny III	8,885 cd	9,960	Yellow Summer	Transgenic
Prelude II	8,443 cd	9,465	Yellow Summer	Transgenic
Supersett	8,047 cd	9,021	Yellow Summer	Precocious Yellow
Dixie	7,411 cd	8,308	Yellow Summer	Susceptible
Puma	6,352 d	7,121	Zucchini	Interspecific
Mulch				
Silver	10,633	11,920		
Black	8,391	9,406		
Probabilities				
Variety	0.000			
Mulch	0.001			
Variety × mulch	0.098			

[z]Means followed by the same letter within the column are not different by Fisher's protected LSD ($P \leq 0.05$).

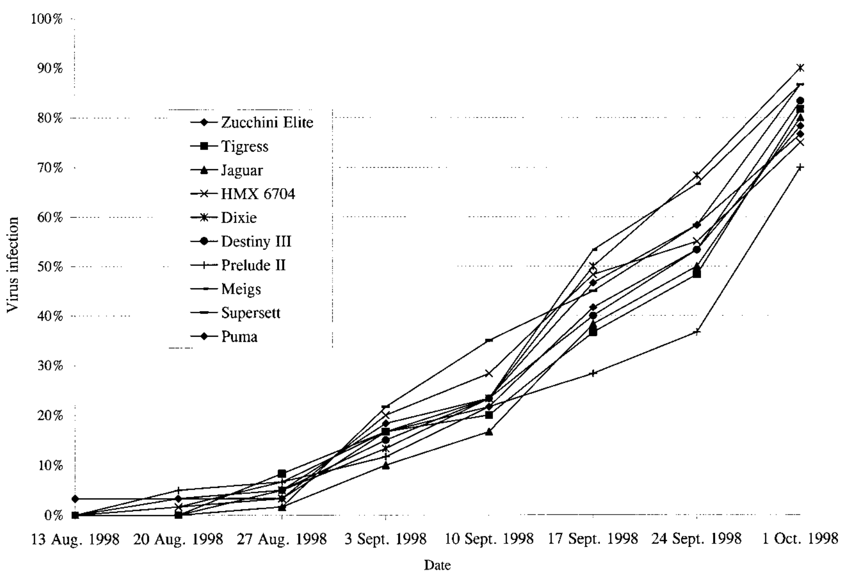

Fig. 3. Percent virus infection over time by variety in Shorter, Ala. (Expt. 4).

vere infection compared to 'HMX 7710', 'HMX 6704', 'Puma', 'Tigress', 'Prelude II', 'Jaguar', and 'Destiny III' (Table 6). In addition, 'Meigs' and 'Dixie' had significantly less infection than 'Zucchini Elite' and 'Supersett'.

Discussion

Variety played a primary, but inconsistent role in yield response differences. No variety whether yellow or zucchini type consistently had the best yield over time or location.

Three different types of reflective surfaces were used in these experiments. Although light reflectance was not measured, there were differences in reflectivity among these surfaces. Certainly the Colorup product, which is a silvered plastic, was the most reflective followed by the aluminum paint and then the aluminum roof coating. Surprisingly, differences for virus incidence occurred with the aluminum paint at the Shorter location, but did not occur with the silvered plastic at the Shorter location. This is probably more a function of disease pressure rather than the performance of the product. There was a much greater disease pressure during the experiment with the silvered plastic compared to the aluminum paint.

Mulch type played a role only in Expt. 4, where reflective mulch had higher yields. In Expt. 1, where reflective mulch played a role in lowering virus incidence, it did not result in greater yields. These results contradict other work with reflective mulches (Brown et al., 1993, 1996). This work, as with previous work, indicates reflective mulch only delays the onset of virus symptoms (Brown et al., 1993, 1996), therefore, the lack of correlation between virus incidence and yield response should not be considered unusual. In Expt. 2, the variety by mulch interaction was a type of nonpreference interaction where the yield response changes with each vari-

Table 5. Percent virus infection based on variety, 5 Oct. 1998, Shorter, Ala. (Expt. 4).

Variety	Virus incidence[z] (%)
Dixie	90 a
Meigs	87 ab
Supersett	87 ab
Destiny III	83 abc
Tigress	82 abc
Jaguar	80 bc
Zucchini Elite	78 bcd
Puma	77 cd
HMX 6704	75 cd
Prelude II	70 d

[z]Means followed by the same letter within the column are not different by Fisher's protected LSD ($P \leq 0.05$).

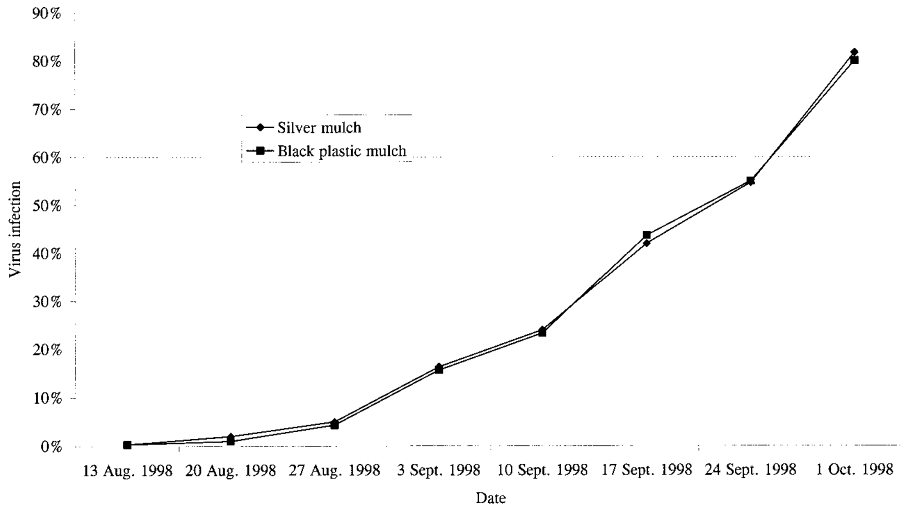

Fig. 4. Percent virus infection over time by mulch type in Shorter, Ala. (Expt. 4).

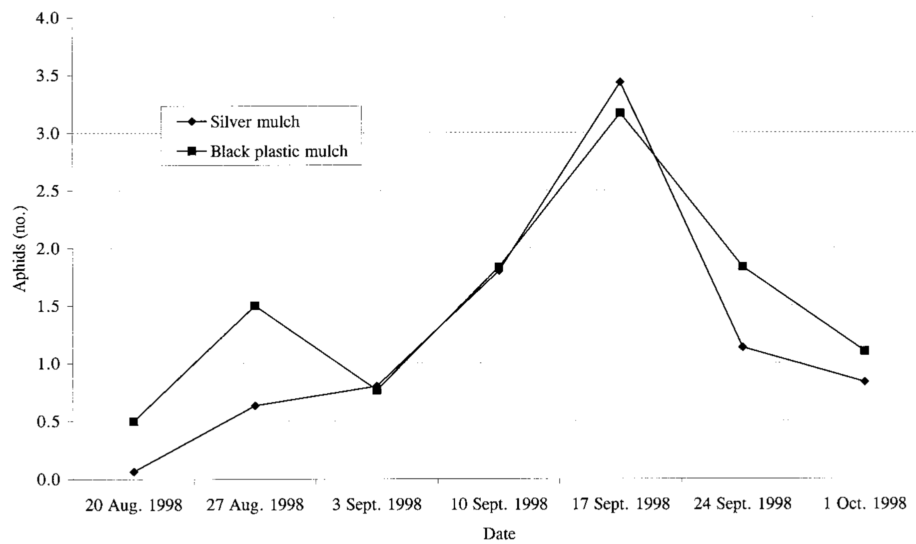

Fig. 5. Aphid count averages for two leaves based on mulch type in Shorter, Ala. (Expt. 4).

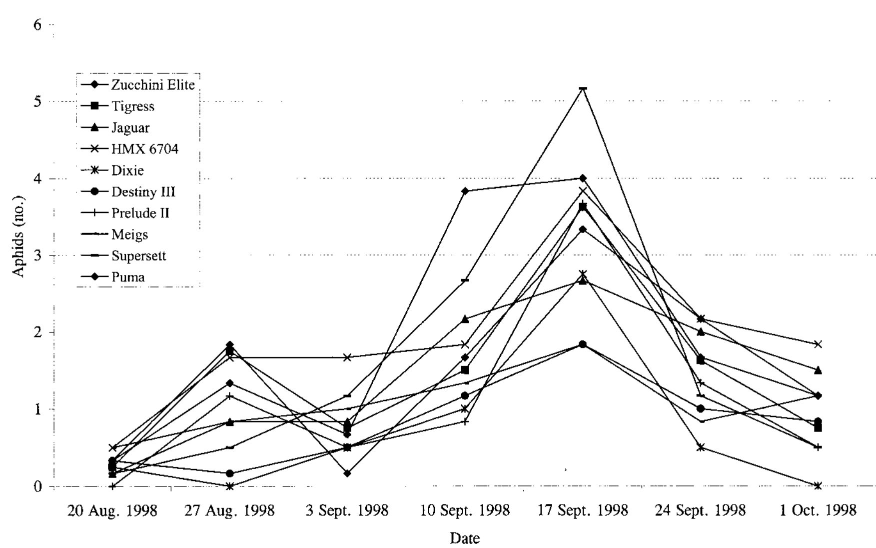

Fig. 6. Aphid count averages for two leaves by variety over time, Shorter, Ala. (Expt. 4).

ety–mulch combination. This type of interaction along with no interaction in other experiments reinforces the idea that synergistic or antagonistic interactions do not occur between the variety and mulch factors.

New transgenic varieties have incorporated resistance to more than one virus by using coat protein genes from different viruses. However, because squash are susceptible to so many different viruses, these varieties often show virus symptoms particularly later in the season, presumably to viruses whose coat protein genes have not been incorporated. As coat protein genes from other viruses are incorporated into squash varieties, it will be interesting to see the outcome. Will we achieve full season complete resistance or will this expose still other unknown viruses? The incorporation of new coat protein genes may also accelerate the selection of new resistant virus strains.

Under low virus pressure as occurred in 1996 at the Shorter location the usefulness of both reflective mulches and resistant varieties is more evident, but under the higher virus pressure of 1998 the usefulness of these techniques to control viruses breaks down. Neither reflective nor black plastic mulch was effective under the high virus pressure of 1998. Although there were differences based on variety, having 70% vs. 90% infection offers very little practical benefit.

In conclusion, viruses will continue to be a problem for squash growers particularly during late season pro-

Table 6. Evaluation of varieties infected with zucchini yellow mosiac virus under greenhouse conditions (Expt. 5).

Variety	Rating[z,y]
Zucchini Elite	5.0 a
Supersett	4.5 a
Meigs	3.1 b
Dixie	2.9 b
HMX 7710	0.2 c
HMX 6704	0.2 c
Puma	0.0 c
Tigress	0.0 c
Prelude II	0.0 c
Jaguar	0.0 c
Destiny III	0.0 c

[z]Rating: 0 to 5 scale, 0 = no symptoms, 5 = severe symptoms.
[y]Means followed by the same letter in the column are not different by Fisher's protected LSD ($P \leq 0.05$).

duction. Reflective mulch offers only part season control of viruses under low virus pressure. Under high virus pressure, reflective mulches do not appear to be effective. Resistant varieties performed as expected under greenhouse conditions, but this did not translate into field performance. Resistant varieties did not consistently perform better than nonresistant varieties whether viruses were present or not.

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