section containing the identification keys. As the intended market for this program includes non-entomologists, this section contains many links to definitions and drawings explaining and illustrating the specific structures. Identification keys are given in both plain text and hyperlinked graphical formats. In the graphical key, the user is presented with paired drawings clearly illustrating the key couplet. By clicking on the appropriate picture, the next couplet is presented. This process is repeated until an identification is made. Each species appearing in both the textual and graphical keys is linked to the appropriate species description.

The final sections provide information on scouting techniques and considerations, trapping methods, and specimen preservation. Scouting and sampling recommendations are derived from GCC's field experiences. Preservation techniques are presented covering field to laboratory preservation, and the preparation of microscope slide mounted specimens. A list of suppliers accompanies sections where specialized materials are mentioned.

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Proc. Fla. State Hort. Soc. 110:299-302. 1997.

EVALUATION OF VIRUS-RESISTANT SQUASH VARIETIES

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Additional index words. Transgenic, watermelon mosaic virus 2, zucchini yellow mosaic virus, *Cucurbita pepo.*

Abstract. **Aphid-borne viruses affecting cucurbits have been a perennial problem for squash (*Cucurbita pepo* L.) and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] growers in Florida. Insecticides have been of little or no value in reducing virus spread, and other methods, such as mineral oil sprays, have not been totally effective. Recently, varieties with resistance derived from the incorporation of the coat protein genes of watermelon mosaic virus 2, zucchini yellow mosaic virus, and cucumber mosaic virus have become commercially available. At the same time, zucchini varieties with interspecific resistance or tolerance to one of more of these three viruses have also been released. We evaluated several of these varieties at the Central Florida Research and Education Center in Leesburg in 1996 in order to compare yield and horticultural traits with standard yellow summer squash and zucchini varieties. The transgenic yellow squash varieties 'Prelude II', 'Destiny III', and 'Liberator III' outperformed 'Dixie' at the end of the growing season when virus pressure was high. 'Prelude II' was the most acceptable variety horticulturally. The resistant zucchini varieties 'Dividend', 'Revenue', and 'Tigress' performed well compared with 'Zucchini Elite'. 'Jaguar' and 'HMX5728' (now 'Puma') produced significantly fewer fruit than the other zucchini varieties both in spring and fall trials. Yield differences among zucchini varieties due to viral infection were not detected.**

Squash (*Cucurbita pepo* L.) crops in Florida suffer regular yield losses due to infection with watermelon mosaic virus 2 (WMV 2), zucchini yellow mosaic virus (ZYMV), and the watermelon strain of papaya ringspot virus (PRSV-W). Cucumber mosaic virus (CMV) is not found as often but is also potentially a threat to cucurbit production in Florida. Until recently, resistant varieties have not been commercially available, and other control measures aimed at interfering with transmission by the aphid vectors, such as stylet oil (Webb and Linda, 1993) have not been totally effective. Floating row covers are very effective barriers to virus transmission by aphids (Webb, 1991) but are expensive to use. Insecticides are of little value because insect transmission of these viruses can occur in a matter of seconds during very brief probes of the plant epidermis.

The need to have resistance to several viruses has made it difficult to develop resistant varieties by conventional methods. Only recently, five zucchini varieties with varying levels of interspecific resistance to ZYMV, WMV 2, and CMV have been released after decades of effort. For a number of plant viruses, however, it has been found that incorporating the viral coat protein gene into the plant DNA protects the plant from infection by the virus (Beachy et al., 1990). Several squash varieties have been developed that have pathogen-derived resistance to two or three of the four viruses that commonly infect cucurbits (Tricoli et al., 1995).

Trials were conducted in the spring and fall of 1996, at the Central Florida Research and Education Center in Leesburg, to compare virus resistance and yield of squash varieties that were either genetically engineered to contain the coat protein genes of two or three different viruses or conventionally

Florida Agricultural Experiment Station Journal Series No. N-01490.

bred for resistance to these viruses using interspecific sources of resistance. In addition, a variety with the precocious yellow character, shown to mask the expression of symptoms of WMV 2 infection in fruit (Adlerz et al., 1985), was included in the spring trial. Preliminary reports of this work have been published (Tyson and Webb, 1997a; 1997b).

Materials and Methods

Spring. On 18 Mar., 'Prelude II', 'Dixie', 'Zucchini Elite', 'Jaguar', 'Tigress', 'HMX5728', and 'Dividend' were direct-seeded in a randomized complete block design with four replications. Each plot (replication) consisted of two rows, 5 ft apart, with nine hills spaced 2.5 ft apart within each row. Plants were thinned to one plant per hill. Soil type was Alachua fine sand and all cultural practices followed University of Florida Extension recommendations. Overhead irrigation was used to supplement rainfall. Fungicide (either mancozeb or chlorothalonil at a rate of 1.5 lb [a.i.]/acre) was applied weekly, beginning on 24 May. *Bacillus thuringiensis* was applied once (24 May) for pickleworm control. A second trial was planted on 2 Apr. and consisted of five yellow squash varieties: 'Lemondrop L', 'Liberator III', 'General Patton', 'Dixie', and 'Destiny III'. Because the transgenic varieties 'Liberator III' and 'Destiny III' had not been deregulated at the time of the trial, all fruit was destroyed after harvest, as was any squash growing within 30 ft of the trial. Table 1 shows the variety, type, source, and nature of resistance for both trials.

Harvest of the first trial began on 3 May and of the second, on 8 May. Harvest continued, three times per week, until 5 June. Virus incidence was recorded on 20 and 30 May. Leaf samples from 20 plants per variety were collected for testing with double antibody sandwich ELISA using specific antisera to WMV 2, ZYMV, and PRSV-W (supplied by D. E. Purcifull, University of Florida, Plant Pathology Dept., Gainesville, FL) on 4 June.

Fall. The zucchini varieties planted in the spring were also planted in the fall with the addition of another Rogers variety, 'Revenue'. Of the yellow squash varieties, only 'Dixie', 'Prelude II', and 'Destiny III' were included. Squash was direct-seeded on 20 Aug. Experimental design and cultural practices were the same as in the spring. Squash was picked 13 times, beginning on 25 Sept. and finishing on 23 Oct. Fruits were categorized as marketable, culled for undesirable horticultural characteristics, or culled for virus symptoms, and then counted and weighed. All plants in all plots were evaluated for symptoms of viral infection on 19 Sept., 26 Sept., 3 Oct., and 25 Oct.

Table 1. Squash varieties evaluated in spring trials.

Variety	Type	Source	Resistance
Prelude II	YCN[z]	Asgrow	Transgenic, WMV 2, ZYMV
Dixie	YCN	Asgrow	None
Zucchini Elite	Zucchini	Harris Moran	None
Jaguar	Zucchini	Harris Moran	Tolerance to ZYMV
Tigress	Zucchini	Harris Moran	Tolerance to ZYMV
HMX 5728	Zucchini	Harris Moran	Tolerance to ZYMV
Dividend	Zucchini	Rogers Seed	WMV 2, ZYMV, CMV
Lemondrop L	YSN[y]	Asgrow	None
Liberator III	YSN	Asgrow	Transgenic, WMV 2, ZYMV, CMV
General Patton	YSN	Asgrow	Precocious yellow gene
Destiny III	YCN	Asgrow	Transgenic, WMV 2, ZYMV, CMV

[z]Yellow crookneck.
[y]Yellow straightneck.

Table 2. Incidence of virus symptoms in yellow summer squash.

		Percentage of plants with symptoms[z]	
Variety	Type of resistance	20 May	30 May
Lemondrop L	None	3.0 ± 6.0[y]	68.3 ± 23.8
General Patton	Precocious yellow gene	4.3 ± 8.5	73.3 ± 21.1
Dixie	None	10.0 ± 12.3	64.0 ± 16.2
Liberator III	Transgenic: CMV, WMV 2, ZYMV	0.0	1.5 ± 3.0
Destiny III	Transgenic: CMV, WMV 2, ZYMV	0.0	0.0
Prelude II	Transgenic: WMV 2, ZYMV	0.0	0.0

[z]Mean of four replications, 18 plants per replication.
[y]Mean ± standard deviation.

Table 3. Incidence of virus symptoms in zucchini squash.

		Percentage of plants with symptoms[z]	
Variety	Type of resistance	20 May	30 May
Zucchini Elite	None	0.0	22.3 ± 20.8[y]
Jaguar	ZYMV tolerance	0.0	4.5 ± 3.0
Tigress	ZYMV tolerance	0.0	4.3 ± 5.3
HMX 5728	ZYMV tolerance	0.0	4.3 ± 5.3
Dividend	WMV 2, ZYMV, CMV	0.0	1.5 ± 3.0

[z]Mean of four replications, 18 plants per replication.
[y]Mean ± standard deviation.

Results and Discussion

Spring. Plants did not show symptoms of virus infection until late in the growing season (Tables 2 and 3). Incidence of symptoms increased rapidly in susceptible yellow squash varieties between 20 May and 30 May. Only one or two plants of the transgenic variety 'Liberator III' showed symptoms of infection; immunoassays showed the presence of both WMV 2 and ZYMV. Zucchini varieties did not show the high incidence of symptoms observed among the yellow squash variet-

Table 4. Viruses identified from squash by DAS-ELISA, June 1996.

		Plants with virus (%)[z]	
Variety	Type	WMV 2	ZYMV
Lemondrop L	Yellow straightneck	65	65
Liberator III	Yellow straightneck	5	10
General Patton	Yellow crookneck	95	60
Dixie	Yellow crookneck	65	35
Destiny III	Yellow crookneck	0	0
Prelude II	Yellow crookneck	0	0
Zucchini Elite	Zucchini	45	10
Jaguar	Zucchini	40	0
Tigress	Zucchini	10	0
HMX 5728	Zucchini	10	0
Dividend	Zucchini	0	0

[z]Twenty samples per variety were collected on the basis of symptoms (randomly from varieties with no symptoms) and tested by double antibody sandwich ELISA. No PRSV-W was found; CMV was not included in tests. Percentages do not reflect overall incidence of infection but indicate which viruses were present and in what proportion.

Table 5. Mean total number and weight of squash fruits per plant, Trial 1, spring 1996.

	Marketable fruits	Marketable fruits	Cull fruits[z]	Cull fruits	Fruits with virus symptoms	
Variety	(no.)	(lb)	(no.)	(lb)	(no.)	(lb)
Zucchini Elite	15.2 b	10.3 a	1.4 c	1.3 d	0.0 b	0.0 b
Tigress	15.0 b	10.9 a	2.0 c	2.0 b	0.0 b	0.0 b
Dividend	12.8 b	8.2 b	2.0 c	1.4 cd	0.0 b	0.0 b
Jaguar	10.2 c	7.3 bc	3.0 b	2.6 a	0.0 b	0.0 b
HMX 5728	8.4 c	6.6 c	1.9 c	1.8 bc	0.0 b	0.0 b
Prelude II	20.4 a	7.8 bc	4.0 a	2.0 b	0.0 b	0.0 b
Dixie	20.3 a	7.6 bc	4.0 a	1.7 bcd	1.4 a	0.6 a

Mean separation in columns by Waller Duncan *k*-ratio test, *k* = 100 (ca. 5% level).
[z]Fruit culled because of undesirable horticultural characteristics.

ies, but the susceptible 'Zucchini Elite' was more affected than the resistant varieties.

ELISA results showed a similar incidence of WMV 2 and ZYMV in susceptible yellow squash varieties with many plants infected with both viruses (Table 4). Only 10% of 'Zucchini Elite' plants and none of the ZYMV-tolerant varieties tested were infected with ZYMV. 'Jaguar' was as susceptible to WMV 2 as 'Zucchini Elite'. Only 'Dividend', with resistance to WMV 2, ZYMV, and CMV, was free of virus. No plants were infected with PRSV-W, a virus more commonly found in the fall in Central Florida.

Of the zucchini varieties, 'Zucchini Elite', 'Tigress', and 'Dividend' produced similar numbers of squash (Table 5). Although 'HMX 5728' was a very vigorous plant, it did not produce as much squash as 'Tigress', 'Zucchini Elite', or 'Dividend'. There were no differences in yield that could be attributed to viral infection.

The incidence of virus was low for most of the growing season. Differences in yield that could be attributed to resistance were only apparent among the yellow squash varieties in the fourth week of harvest (Fig. 1). Season totals showed no difference between the susceptible 'Dixie' and the transgenic 'Prelude II' (Table 5) or between 'Dixie' and 'Destiny III'

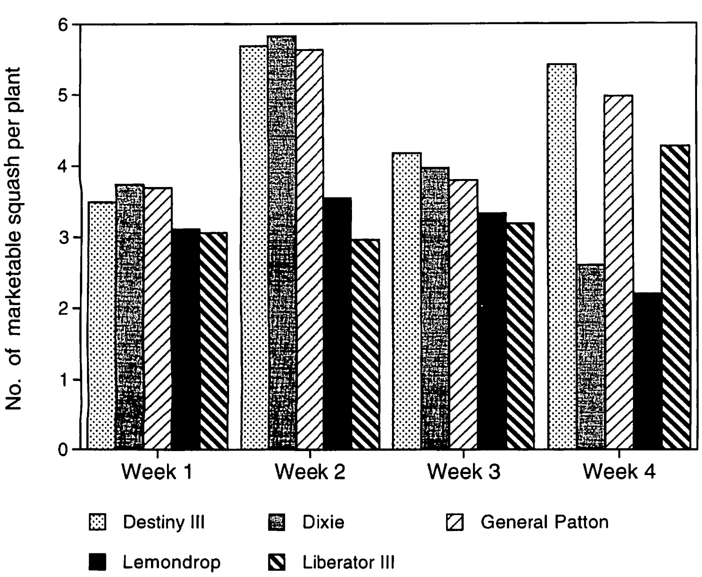

Figure 1. Number of marketable yellow summer squash per plant by week, spring trial.

Table 6. Mean total number and weight of squash fruits per plant, Trial 2, spring 1996.

	Marketable fruits	Marketable fruits	Cull fruits[z]	Cull fruits	Fruits with virus symptoms	
Variety	(no.)	(lb)	(no.)	(lb)	(no.)	(lb)
Destiny III	18.8 a	6.3 ab	4.9 a	2.5 a	0.0 b	0.0 b
General Patton	18.1 a	6.7 a	2.2 b	1.2 b	1.3 b	0.7 ab
Dixie	16.1 ab	5.5 bc	3.9 a	1.9 ab	4.3 a	3.4 a
Liberator III	13.5 bc	5.7 bc	2.4 b	1.5 b	0.0 b	0.0 b
Lemondrop	12.2 c	4.9 c	2.1 b	1.1 b	3.6 a	1.6 ab

Mean separation in columns by Waller Duncan *k*-ratio test, *k* = 100 (ca. 5% level).
[z]Fruit culled because of undesirable horticultural characteristics.

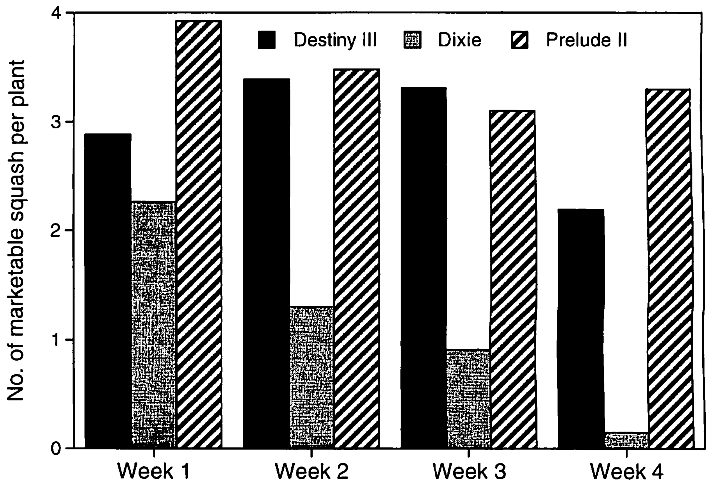

Figure 2. Number of marketable yellow summer squash per plant by week, fall trial.

(Table 6). Similarly, there were no differences between the susceptible straightneck variety 'Lemondrop' and the transgenic 'Liberator III'. Differences in yield were assumed to be due to varietal differences. 'General Patton', which has the precocious yellow character, produced significantly more squash by weight than 'Dixie' and had fewer fruits with symptoms of viral infection.

Fall. Virus incidence was much higher in the fall, reaching 87% in the susceptible 'Dixie' by 3 Oct. The effects of viral infection were evident in the decrease in yield of 'Dixie' over

Table 7. Mean total number and weight of squash fruits per plant, fall 1996.

	Marketable fruits	Marketable fruits	Cull fruits[z]	Cull fruits	Fruit with virus symptoms	
Variety	(no.)	(lb)	(no.)	(lb)	(no.)	(lb)
Revenue	9.9 b	5.5 a	0.9 bc	0.5 abc	0.0 b	0.0 b
Dividend	9.6 b	5.2 a	1.1 bc	0.6 abc	0.0 b	0.0 b
Tigress	9.0 b	5.4 a	1.1 bc	0.6 ab	0.0 b	0.0 b
Zucchini Elite	8.5 b	5.3 a	0.9 bc	0.6 abc	0.5 b	0.3 b
Jaguar	5.5 c	3.2 cd	1.1 bc	0.8 a	0.0 b	0.0 b
HMX 5728	4.4 c	2.6 de	0.7 c	0.4 bc	0.0 b	0.0 b
Prelude II	14.4 a	4.5 ab	1.3 b	0.4 bc	0.0 b	0.0 b
Destiny III	12.2 a	3.7 bc	1.9 a	0.7 a	0.0 b	0.0 b
Dixie	5.2 c	1.8 e	0.7 c	0.3 c	5.4 a	1.5 a

Mean separation in columns by Waller Duncan *k*-ratio test, *k* = 100 (ca. 5% level).
[z]Fruit culled because of undesirable horticultural characteristics.

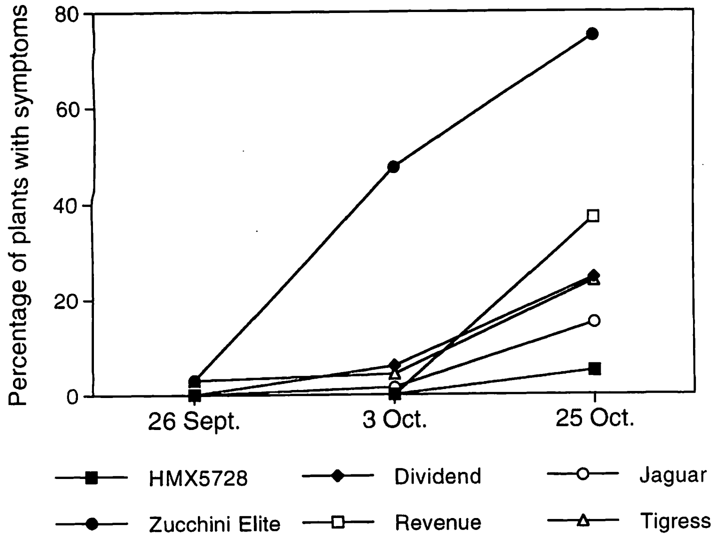

Figure 3. Percentage of zucchini squash plants showing symptoms of viral infection, fall trial.

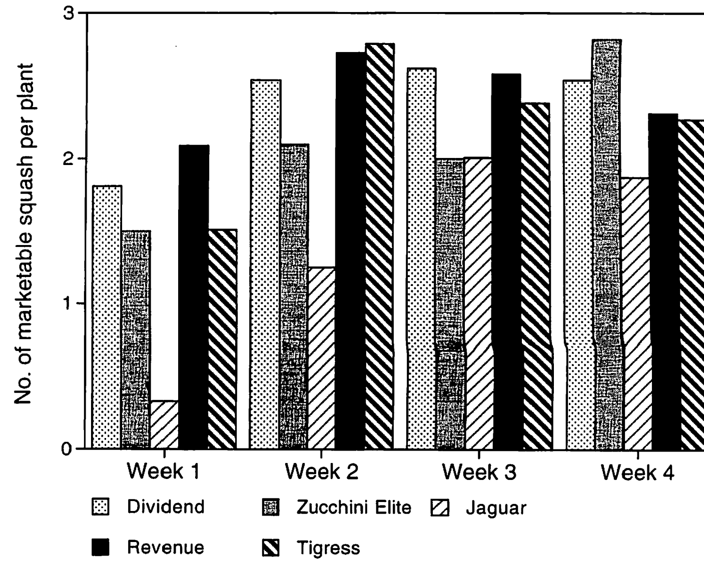

Figure 4. Number of marketable zucchini squash per plant by week, fall trial.

the season (Fig. 2), and in seasonal totals (Table 7). In contrast, the transgenic 'Prelude II' produced as much marketable squash as the best zucchini varieties. 'Destiny III' had more undesirable horticultural characteristics in the fall, including double squash, variation in curvature, and development of green peduncle tissue along the full length of the fruit.

Differences in susceptibility to viral infection among the zucchini varieties were more obvious in the fall (Fig. 3). Although no variety was completely resistant, all those with some resistance showed symptoms much later than 'Zucchini Elite'. 'Jaguar' and 'HMX 5728' showed the lowest level of infection at the end of the season, but produced significantly fewer fruit than the other four varieties. These varieties flowered later and produced very little fruit during the first week of harvest (Fig. 4, data for 'HMX 5728' not shown).

In our trials and in trials elsewhere in Florida (Hochmuth et al., 1997; White, 1997) as well as in Oregon and New York (Clough and Hamm, 1995; Fuchs and Gonsalves, 1995) transgenic squash containing viral coat protein genes performed as well or better than standard yellow squash varieties. We now have, for the first time, a highly effective way to control the most important aphid-borne viruses affecting yellow summer squash. Zucchini varieties with tolerance or resistance to one or more of these viruses also performed well and provide good alternatives to highly susceptible varieties.

These varieties will be particularly useful for growers in Central and North Florida. At this time, there are no squash varieties with either transgenic or interspecific resistance to PRSV-W, the most important virus affecting cucurbits in South Florida (Purcifull et al., 1988).

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