Variety Trials

Yields and Disease Resistance of Fall-harvested Transgenic and Conventional Summer Squash in Kentucky

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ADDITIONAL INDEX WORDS. biotechnology, coat protein, *Cucurbita pepo*, downy mildew, *Erysiphe cichoracearum*, papaya ringspot virus, powdery mildew, precocious yellow, *Pseudoperonospora cubensis*, *Sphaerotheca fuliginea*, watermelon mosaic virus, yellow squash, zucchini, zucchini yellow mosaic virus

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University of Kentucky Agricultural Experiment Station Publication no. 98-11-184. The authors gratefully acknowledge the hard work and technical assistance of Darrell Slone, Janet Pfeiffer, Dave Lowry, Dave Spalding, and Jason Whitis together with financial support from participating seed companies. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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SUMMARY. Virus and fungal disease pressures limit fall production of summer squash (*Cucurbita pepo* L.) in Kentucky. Twenty-five summer squash cultivars (nine zucchini, eight yellow straightneck, and eight yellow crookneck entries) were evaluated for marketable yield, appearance, and disease resistance in a late summer planting. Genetically engineered virus-resistant materials and new conventionally bred resistant or tolerant cultivars were compared with popular susceptible hybrids. Virus incidence was determined visually before and after final harvest and was also determined by enzyme-linked immunosorbent assay (ELISA). Watermelon mosaic virus (WMV) was most frequently detected and appeared to have caused most of the observed symptoms. Conventionally bred cultivars containing the precocious yellow gene and two transgenic lines were in the highest yielding group of yellow straightneck squash despite high virus incidence in precocious yellow cultivars. Among yellow crooknecks, transgenic cultivars were clearly superior for disease resistance and yields. Conventionally bred cultivars with virus tolerance were among the highest yielding zucchini types. Most transgenics were superior to their nontransformed equivalent cultivars for virus resistance and yield. Cultivars and breeding lines varied considerably in color, shape, and overall appearance. ELISA results revealed that some (but not all) transgenic cultivars tested positive for the coat protein corresponding to the virus resistance present in that cultivar. Also, mild virus-like symptoms were observed in transgenic squash plants after the conclusion of harvest.

With the ongoing consolidation of production and persistent uncertainties surrounding tobacco cultivation in the United States, an increasing number of Kentucky tobacco growers are looking for new opportunities in commercial vegetable production. Extensive studies in the mid-1980s identified market windows and crops for which Kentucky might have comparative advantages in the national marketplace; summer squash (yellow straightneck, yellow crookneck, and zucchini types) was rated highest among 21 vegetable crops examined for their Kentucky production and marketing potential. Although squash

from late summer plantings was cited as having most favorable prices, virus diseases caused serious damage in fall-harvested squash and was an important barrier to profitable production (Snyder, 1991).

Mixed virus infections commonly occur in fall-harvested summer squash in Kentucky. Watermelon mosaic virus (WMV, formerly WMV-2), zucchini yellow mosaic virus (ZYMV), squash mosaic virus (SqMV), cucumber mosaic virus (CMV) and papaya ringspot virus (PRSV, formerly WMV-1) have all occurred in Kentucky at one time or another and in most other southeastern states. Although the dominant virus(es) vary from site to site and from year to year, WMV has been the most frequently detected virus in summer squash (present in >90% of samples tested) during the last 8 years in Kentucky (W. Nesmith, unpublished). Virus epidemics are often severe in late plantings and total destruction of the crop has been fairly common. WMV has also been reported as the principal cause of virus disease in middle- to late-season plantings in Mississippi (Snyder et al., 1993). As much as 70% of the crookneck squash crop in Georgia has been damaged by virus diseases; some Georgia growers plant 50% more than they expect to harvest to make up for losses from virus diseases (Balaban, 1996).

All of the above-mentioned viruses, with the exception of SqMV, are aphid-transmitted in a nonpersistent manner; insecticidal control of aphids has therefore not been an effective means of reducing the amount of virus in late plantings of summer squash. Recommended cultural control practices such as eliminating broadleaf weeds around the crop, using mineral oils, and planting on aluminum foil mulches may delay virus infections in summer squash. Unfortunately these measures have rarely been put into practice in the state and may not provide adequate control in late plantings when aphid populations are high and disease pressure is most intense.

Although several fall-harvested summer squash trials had been conducted in Kentucky, no virus resistant cultivars were available at the time of those trials. Yellow squash harvests were sometimes abandoned due to greening of fruit caused by virus diseases (Roberts et al., 1988). Most yellow straightneck squash growers in

Kentucky depend on cultivars with the precocious yellow (*Py*) gene for late season production. These cultivars mask the greening effect in summer squash fruit if plants are infected by CMV or WMV but not if plants are infected by PRSV or ZYMV. In some areas, precocious yellow squash may be more difficult to market because of buyer preference for green, rather than yellow fruit peduncles found in cultivars with the *Py* gene (Snyder et al., 1993; J. Schultheis, personal communication). Production of yellow crookneck squash, grown primarily in the southern part of Kentucky, has been extremely risky in the absence of virus resistant or tolerant cultivars.

New summer squash cultivars and breeding lines claiming resistance to two or more cucurbit viruses recently became available for widespread field testing. These cultivars could perhaps significantly reduce the risk associated with squash production in Kentucky and other states. Asgrow Seed (subsidiary of Seminis Vegetable Seeds) developed these new plant materials through genetic engineering or a combination of genetic engineering and traditional plant breeding techniques (D. Tricoli and D. Groff, personal communication). Clones of the genes producing coat proteins of these viruses were transferred to a squash plant genome by means of a disarmed bacterial vector (Quemada, 1996; Tricoli et al., 1995). The first transformations were performed on a parent inbred of a popular hybrid yellow crookneck cultivar; plants were regenerated by tissue culture to reproduce the same inbred but with the viral coat protein gene(s) added. This inbred was then used in its original hybrid combination to produce a virus-resistant F_1 cultivar. Since these gene constructs for one or more viral coat proteins are inherited as a single dominant trait (Tricoli et al., 1995), multiple virus resistance could be easily incorporated into noncrookneck squash cultivars using traditional breeding techniques. The goal has been to obtain hybrid cultivars horticulturally identical or nearly identical to the original but with coat protein genes to confer virus resistance in the field. Asgrow has used viral coat protein genes for resistance to WMV, ZYMV, and CMV together with conventional breeding methods to add PRSV resistance to yellow and zucchini squash.

Asgrow received FDA approval for two transgenic yellow crookneck squash cultivars in 1995 when 'Freedom II' became the first commercially available plant cultivar with a transgenic source of disease resistance. Although results from the first field trials looked very promising in terms of virus resistance and yields (Arce-Ochoa et al., 1995; Clough and Hamm, 1995; Fuchs and Gonsalves, 1995), production-scale trials of 'Freedom II' (transformed 'Pavo' with WMV and ZYMV resistance) in Georgia resulted in a lower than expected packout (Balaban, 1996). Grower disappointment was apparently due to factors unrelated to the transgenic resistance of 'Freedom II' or the transformation process; intense whitefly pressure and the prevalence of PRSV contributed to the poor performance of this cultivar in Georgia in 1995. Part of the problem with 'Freedom II' was that little commercial production-level field testing could be undertaken before the release of the cultivar given the strict USDA-APHIS access and containment regulations in place at the time (R. Hernandez and D. Groff, personal communication). Asgrow advertised other new yellow squash cultivars claiming multiple virus resistance from genetic engineering and/or conventional breeding in Winter 1996 and one of these ('Prelude II') came on the market in 1997.

Transgenic cultivars and breeding lines from Asgrow along with conventional cultivars were evaluated for yield and virus resistance in North Carolina in 1995 and 1996 (Schultheis and Walters, 1998). These tests included a spring trial and two fall trials (one of which was destroyed by hurricane after the first harvest). We wanted to evaluate these transgenic and other new squash cultivars for yield and potential marketability in Kentucky using black plastic mulch and drip irrigation in a fall trial when viral disease pressure would be severe. In addition to evaluating cultivars and breeding lines for yield and virus resistance, we also evaluated entries for tolerance to powdery mildew (*Sphaerotheca fuliginea* and *Erysiphe cichoracearum* were present), and downy mildew (*Pseudoperonospora cubensis*). Preliminary results were published earlier for the benefit of growers and others who could make immediate use of this information (Rowell et al., 1997, 1998).

Materials and methods

Twenty-five summer squash cultivars or advanced breeding lines (nine zucchini, eight yellow straightneck, and eight yellow crookneck entries) were evaluated at the University of Kentucky Horticultural Research Farm in Lexington in the late Summer and Fall 1997. Cultural practices were according to our current commercial recommendations for Kentucky. Seeds were sown in the greenhouse on 18 July in 72-cell plastic trays and transplanted to the field on 5 Aug. Each plot consisted of eight plants spaced 18 inches (46 cm) apart in a single row on 6-inch (15-cm) high raised beds with black plastic mulch and trickle irrigation. Beds were 6 ft (1.8 m) apart from center to center. All 25 entries were planted together in a randomized complete block design with four replications. Each block consisted of two rows with 12 or 13 entries per row. A long single row of the virus-susceptible 'Pavo' was planted between each block and on the outside edges of the field to enhance natural virus buildup and uniform spread throughout the trial. Because of poor germination and insufficient numbers of plants of some entries, data from the fourth replication were omitted from statistical analyses.

In total, 150 lb/acre (168 kg·ha⁻¹) of preplant and fertigated nitrogen was applied. All P and K was applied preplant according to recommendations based on soil tests. Mancozeb (Dithane) or chlorothalonil (Bravo) protective fungicides were applied weekly and one application of triadimefon (Bayleton) was made shortly after the appearance of powdery mildew in the field. All fungicide applications were discontinued after 10 Sept. to allow development of fungal diseases late in the trial. Esfenvalerate (Asana XL) was applied on an as-needed basis to control cucumber beetle and squash vine borer.

Plots were harvested on different days six times per week from 26 Aug. until mid-September and three to four times per week thereafter until 3 Oct. for a total of 24 harvests. Fruit were counted and weighed after grading into either marketable fruit or culls. Marketable yield was expressed in terms of the number of half-bushel boxes per acre by dividing the total weight of marketable fruit per plot by 21 lb

(9.52 kg). When more than half of the fruit harvested from a plot showed virus symptoms, the plot was flagged and harvesting of that plot discontinued. Following an analysis of variance, means of all variables were compared using Waller-Duncan's K ratio *t* test ($P = 0.05$).

Several comparisons between groups of transgenic and conventional squash cultivars were planned before conducting the trial. Among the 25 cultivars and breeding lines tested, our trial included four pairs of transgenics and their nontransformed equivalent cultivars: 'Liberator III' and 'Lemondrop' (straightnecks), 'Prelude II' and 'Prelude' (crooknecks), 'Destiny III' and 'Dixie' (crooknecks), 'Independence II' and 'Senator' (zucchini). Single degree-of-freedom contrasts were used to compare transgenic and conventional cultivars.

'Whitaker' (NY 247), an open-pollinated cocozelle type summer squash from Cornell University, was included in the trial in an unreplicated observation plot. 'Whitaker' has resistance to ZYMV, CMV, and PRSV obtained through a complex conventional plant breeding scheme involving interspecific hybridization and embryo culture (R. Robinson, personal communication).

FRUIT QUALITY RATINGS. All fruit of each trial entry harvested from one replication were graded and laid out on tables for careful examination and quality rating on 25 and 26 Sept. by two independent evaluators (one evaluator was a local produce wholesaler). Appearance ratings included, in order of importance: overall attractiveness, shape, uniformity of shape, color, and defects.

DISEASE ASSESSMENTS. Virus incidence (percentage of plants showing any virus-like foliar symptoms) was assessed visually on 22 Sept. (10 d before final harvest) for yellow squash and again on 12 Oct. (9 d after final harvest) for yellow and zucchini squash. Virus symptoms were not evident in most of the zucchini cultivars at the first assessment. Leaf samples from three symptomatic plants of each cultivar from each replication were collected on 25 Sept. and 12 Oct. Samples were also taken at random from plots containing no symptomatic plants. All leaf samples were pooled for each cultivar within plots and sent to Agdia, Inc. (Elkhart, Ind.) for ELISA testing.

Samples were assayed for CMV, Impatiens Necrotic Spot Virus, PRSV, Potato Virus Y, SqMV, Tobacco Mosaic Virus, Tomato Mosaic Virus, Tobacco Ringspot Virus, Tomato Ringspot Virus, Tomato Spotted Wilt Virus, WMV, and ZYMV.

Plants were visually assessed for the percentage of upper leaf surfaces showing powdery and/or downy mildew symptoms on 12 Oct. Identification of powdery mildew species was based on microscopic examination of conidia (see Zitter, 1996) from leaf samples collected at random locations within the trial field. It should be emphasized that control of these diseases was excellent for most of the harvest season due to fungicide applications and low humidity. Mildew infections appeared to have little or no impact on yields.

Results and discussion

VIRUS DISEASES, COAT PROTEINS, AND ELISA. Plants of the susceptible cultivar in the guard rows were the first to exhibit foliar virus symptoms during the third week of August (around the first harvest date). Symptoms were evident in most of the susceptible yellow squash cultivars soon thereafter but were not obvious in susceptible zucchini cultivars until much later, toward the end of the harvest period. Although samples from each entry were ELISA-tested for 12 different viruses, only WMV and CMV were detected. WMV was detected in all entries except for transgenic cultivars 'Liberator III', 'Prelude II', and 'Destiny III' (Table 1). Because WMV was the virus most frequently detected, we assume it caused most of the symptoms observed in the field. This assumption is consistent with other samplings for cucurbit viruses recently in the state.

Positive reactions to WMV antiserum occurred in samples from some replications in some of the transgenic cultivars carrying the WMV coat protein gene (XPHT 1816, 'Declaration II', 'Independence II', and XPHT 1777) but not in others. It is possible that these ELISA positives were serological reactions to coat proteins produced by the transgenes rather than from field infections. It is also possible that these reactions were the result of low-level field infections of WMV in the transgenic resistant materials.

Positive reactions to CMV antiserum occurred in all samples taken from

two ('Liberator III' and 'Destiny III') out of the three transgenic cultivars with CMV coat protein resistance but did not occur in any other samples from the trial. Strong symptoms have developed when CMV has occurred in squash in the past; however, there were no CMV symptoms observed in these two cultivars or anywhere else in the trial. In addition, leaf samples from these two cultivars did not produce CMV symptoms when sap was mechanically inoculated onto known CMV-susceptible tobacco plants in the greenhouse. It appears most likely that the antiserum used in the ELISA tests was reacting to CMV coat proteins produced by the transgenes rather than indicating CMV infections in the field. Clough and Hamm (1995) reported that summer squash plants transgenic for WMV and ZYMV coat proteins showed weak positive reactions to WMV and ZYMV antisera. Asgrow researchers confirm that they occasionally see weak serological reactions to viral coat proteins in some transgenic cucurbits (including squash, but especially in melons) but that the coat proteins are not infectious and cause no reaction when inoculated to indicator plants (P. Himmel, personal communication).

ELISA positives apparently occur in samples from some cultivars with transgenic coat proteins but not in others producing the same proteins. We did not attempt to determine exactly why this occurred in this study; however, it is possible that differing levels of expression occurred for the same coat proteins in different cultivars. Expression of viral coat proteins in some transgenic cultivars may have been below detection thresholds in ELISA tests.

YIELDS AND VIRUS INCIDENCE. Conventionally bred hybrids having the precocious yellow gene ('Fortune', 'Multipik', 'Sunbar', and 'Seneca Supreme') were all in the highest yielding group of yellow straightneck squash cultivars in spite of foliar virus incidences ranging from 70% to 100% (first assessment date, Table 1). Plants continued to yield symptomless marketable fruit in spite of severe foliar virus symptoms. Transgenic entries XPHT 1816 and 'Liberator III' were also in this group of highest yielding straightnecks. These results are very similar to those obtained in the fall trial in North Carolina with 'Multipik' (Py)

and 'Liberator III' (Schultheis and Walters, 1998).

Transgenic 'Prelude II' and 'Destiny III' were clearly superior among yellow crookneck entries with marketable yields nearly double those of the lowest yielding crooknecks. Unfortunately no crookneck cultivars with the precocious yellow gene were available to include in the trial. No virus symptoms were observed on any of the transgenic plants at the first assessment date in spite of incidences ranging from 67% to 100% among other cultivars on the same date (Table 1). These results are again very similar to

those reported for crookneck entries in North Carolina although marketable yields of transgenics with resistance to two or three viruses were reduced considerably in that trial by the presence of PRSV (Schultheis and Walters, 1998).

Marketable yields of several of the most popular crookneck hybrids ('Dixie', 'Goldie', 'Pavo', 'Picasso') were greatly reduced by green fruit symptoms associated with virus diseases. Virus incidence was significantly less in 'Prelude II' than in the conventional cultivars on the first and second assessment dates (Table 1). Both 'Pre-

lude II' and its nontransformed equivalent cultivar ('Prelude') were also resistant to powdery mildew (and tolerant to downy mildew in the case of 'Prelude II').

Conventionally bred cultivars claiming virus tolerance ('Dividend', 'Revenue') were among the highest yielding zucchinis (Table 1). Although yields of transgenic zucchini lines were lower than the highest yielding conventional cultivars, these differences were not statistically significant except for 'Independence II', which was lower yielding than most of the other transgenic and conventionally bred

Table 1. Yields and disease assessments for yellow straightneck, crookneck, and zucchini squash cultivars or breeding lines; data are means of three replications, 1997.

Entry	Breeding type ^w	Claimed resistance ^v	Marketable yield boxes/acre ^u	Virus incidence ^y (%)		ELISA ^x		Powdery mildew ^z (%)	Downy ^z mildew (%)	
				22 Sept.	12 Oct.	WMV	CMV			
Yellow straightneck										
Fortune	C-Py	Z,C,W,R	1710	94	80	+	–	67	10	
XPHT 1816	Tg-4		1699	0	73	+	–	10	38	
Multipik	C-Py		1699	70	94	+	–	77	21	
Sunbar	C-Py		1563	79	87	+	–	77	48	
Seneca Supreme	C-Py	Z,C,W	1541	100	100	+	–	92	33	
Liberator-III	Tg-3		1535	0	71	–	+	75	43	
Seneca prolific	C		1199	67	100	+	–	78	80	
Lemondrop-L	C		959	78	67	+	–	73	65	
Yellow crookneck/semicrookneck										
Prelude II	Tg-2	Z,W,P	1599	0	29	–	–	5	42	
Destiny III	Tg-3	Z,C,W,	1571	0	79	–	+	88	80	
Sundance	C		1225	79	100	+	–	47	47	
Prelude	C		1006	83	100	+	–	770		
Dixie	C		898	92	100	+	–	87	70	
Goldie	C		894	95	100	+	–	77	67	
Pavo	C		888	87	100	+	–	57	60	
Picasso	C		836	100	100	+	–	27	92	
Zucchini										
Revenue	C		Z,C,W ^t	2220	–	47	+	–	77	28
President	C	Z,C,W ^t	2170	–	37	+	–	57	30	
Dividend	C		2125	–	75	+	–	95	35	
Zucchini Elite	C		2116	–	53	+	–	72	7	
Declaration II										
(XPHT 1814)	Tg-2	Z,W	2108	–	33	var	–	80	20	
XPHT 1777	Tg-2	Z,W	2040	–	29	var	–	70	4	
Senator	C	Z,W ^t	1923	–	62	+	–	48	25	
Jaguar	C		1783	–	61	+	–	90	25	
Independence II										
XPHT 1776)	Tg-2		Z,W	1589	–	29	var	–	43	10
Waller-Duncan LSD (<i>P</i> = 0.05).			227	25	37			27	41	

^uNumber of half-bushel (21 lb, 9.52 kg) boxes per acre.

^vC = from conventional breeding; Tg = transgenic for resistance to two (Tg-2) or three (Tg-3) viruses; Tg-4 = transgenic for three viruses with resistance to the fourth (PRSV) obtained through conventional breeding; C-Py = conventional breeding incorporating the precocious yellow gene.

^xCompany's descriptions, resistance or tolerance; Z = ZYMV = zucchini yellow mosaic virus, W = WMV = watermelon mosaic virus (formerly called WMV-2), C = CMV = cucumber mosaic virus; R = PRSV = papaya ringspot virus (formerly WMV-1), P = PM = powdery mildew.

^yPercent of plants showing virus-like symptoms; assessed in yellow squash only by BR on 22 Sept. (10 d before end of harvesting); yellow and zucchini assessed by WN on 12 Oct. (9 d after final harvest).

^zResults from testing for 12 viruses by Agdia Inc. of Elkhart, In. (+) Positive reaction in all replications sampled, (-) negative reaction from all replications sampled, var = variable, i.e., positive reactions to samples from some replications, negative from others.

^uPercent of upper leaf surface covered by powdery or downy mildew symptoms; rated 12 Oct (9 d after final harvest and 1 month after final fungicide application).

^tDescribed as tolerant to some strains of these viruses.

zucchini types (Table 1). The only zucchini entry to show virus symptoms on fruit was 'Zucchini Elite'; symptoms first appeared on 22 Sept., ≈ 10 d before the final harvest. Virus symptoms on fruit were not observed in other zucchini cultivars or breeding lines in spite of the incidence of foliar symptoms ranging from 29% to 75% among zucchini entries on the second assessment date.

Yields of 'Whitaker' from one observation plot were good for an open-pollinated cultivar (1552 boxes/acre = 32,592 lb/acre = 36,529 kg·ha⁻¹). No symptoms were evident at the first assessment date; however, all of the plants showed virus-like symptoms at the second assessment date. These symptoms were most likely caused by WMV infections although plants in this plot were not assayed by ELISA. Remarkably little of the leaf surfaces of 'Whitaker' plants showed powdery mildew (0.5%) or downy mildew (10%) symptoms. 'Whitaker' appears to be an excellent source of multiple disease resistance in *C. pepo*. It is available through the Cornell Research Foundation for use as an open-pollinated cultivar or parent line.

Foliar virus symptoms appeared late in the season and were quite mild on transgenic plants. The occurrence of these symptoms was not associated with yield or fruit quality. We were not sure whether these symptoms were a result of low level virus infections in the field or were expression products of the transgenes. Others who have worked with similar materials indicate that low level infections are possible in coat protein transgenic plants given

continuous exposure to the virus(es) in the field (Fuchs and Gonsalves, 1995; R. Grumet, personal communication).

Fuchs and Gonsalves (1995) noted the late season occurrence of mild localized symptoms (small chlorotic dots or splotches on otherwise healthy looking leaves on vigorous plants) in squash with transgenic resistance to WMV and ZYMV. Asgrow researchers have observed similar mild symptoms in the field on artificially inoculated transgenic squash in Michigan during cold weather very early in the season (plants grew out of it after a brief "flash" of symptoms) or at the end of the season when plants were not actively growing (Tricoli et al., 1995; D. Tricoli, personal communication). The presence of WMV and ZYMV could be detected in transgenic resistant plants with these mild symptoms only when symptomatic areas of the leaves were assayed with ELISA (Fuchs and Gonsalves, 1995; Tricoli et al., 1995). Asgrow researchers suspect that changes occur in the effectiveness of coat protein-mediated resistance mechanisms when the plants are not actively growing, permitting low levels of viral replication during such periods. Cooler temperatures prevailed and plants did not appear to be actively growing when our second virus assessment was made on 12 Oct. It seems likely that the symptoms we observed were the result of low level infections under these conditions rather than any expression products of the transgenes.

PLANNED COMPARISONS. Marketable yields were significantly higher and virus incidence on the first assessment date was significantly lower in the group

of four transgenic cultivars compared to their nontransformed equivalents (Table 2). Coat protein resistance reduced early and late virus incidence and improved yields significantly in all transgenic yellow squash cultivars compared to their nontransformed equivalent cultivars ('Liberator III' vs. 'Lemondrop', 'Prelude II' vs. 'Prelude', 'Destiny III' vs. 'Dixie'). Only transgenic 'Independence II' zucchini was significantly lower yielding than its equivalent cultivar 'Senator'. Late virus incidence was lower for the transgenic cultivars (mild symptoms only) compared to their nontransformed equivalents although this difference was not statistically significant.

When the two transgenic straightneck entries were compared with the four conventional precocious yellow straightneck cultivars in the trial, yields were nearly the same in spite of an 86% average virus incidence in the precocious yellow cultivars compared to no symptoms in the transgenics (first assessment date, Table 2). Average virus incidence from the group of transgenic entries was not significantly different from that of the group of *Py* cultivars on the later assessment date; however, only mild symptoms were observed on transgenic plants.

The group of all seven transgenic entries was compared to the entire group of 18 conventional cultivars; transgenics were significantly higher yielding overall (Table 2). Only the late virus assessment could be used to make incidence comparisons between these groups as this was the only date that included the zucchini entries. Late virus incidence was higher in the con-

Table 2. Planned single df comparisons among groups of transgenic and conventional squash cultivars; data are means of three replications, 1997.

Variable	Transgenic	Conventional	Significance
Transgenics (n=4) vs. their nontransformed equivalent cultivars (n=4)			
Yield (boxes/acre ²)	1576	1197	***
Virus incidence ³ (%)	0	84	***
Late virus ^x (%)	52	82	NS
Transgenic straightnecks (n=2) vs. precocious yellow straightneck cultivars (n=4)			
Yield (boxes/acre)	1616	1620	NS
Virus incidence (%)	0	86	***
Late virus (%)	72	91	NS
All transgenics (n=7) vs. all conventional cultivars (n=18)			
Yield (boxes/acre)	1735	1485	***
Late virus (%)	36	81	***

²Half-bushel [21 lb (9.52 kg)] boxes per acre.

³Percentage of plants with foliar symptoms on 22 Sept. Does not include zucchini entries.

^xPercentage of plants with foliar symptoms on 12 Oct.; only mild symptoms on transgenics.

NS,***Nonsignificant at $P = 0.05$ or significant at $P = 0.001$ when compared by single degree-of-freedom contrasts.

Table 3. Fruit color and appearance ratings for yellow straightneck, crookneck, and zucchini squash cultivars or breeding lines; all fruit bulked from three replications harvested on 24 Sept. 1997.

Entry	Type ^z	Color ^y	Rating ^x	Comments
Lemondrop L	SN	2	3	Cylindrical, long, thick neck; ≈50% of fruit slightly curved.
Liberator III	SN	2	4	Cylindrical, long, thick neck; ≈50% of fruit slightly curved.
XPHT 1816	SN	1	3	Cylindrical, long, thick neck; ≈50% of fruit slightly curved; greenish tint.
XPHT 1817	SN	3	5	Cylindrical, long, thick neck; ≈70% of fruit slightly curved; nice color; skin slightly rougher than XPHT 1816.
Sunbar	SN-Py	5	3	Elongated teardrop shape; medium thick neck; ≈70% of fruit w/ noticeable protrusion at blossom end; ≈10% of fruit crescent shaped.
Multipik	SN-Py	5	4	Elongated teardrop shape; medium thick neck; ≈10% of fruit w/ blossom end protrusion; ≈10% to 15% of fruit crescent shaped.
Seneca Supreme	SN-Py	5	5	Elongated teardrop shaped; no blossom end protrusions; <5% crescent-shaped fruit.
Fortune	SN-Py	5	5	Elongated teardrop shaped; medium thick neck; no blossom end protrusions; ≈10% of fruit with slightly curved necks.
Seneca Prolific	SN	2	3	Pale to greenish tint; teardrop but slightly more cylindrical; medium thick neck; ≈50% of fruit with slightly curved neck.
Sundance	CN	3	4	Full CN; very thin necks—easily broken; nice color.
Prelude	CN	2	4	Full CN; thin neck but thicker than ‘Sundance’.
Prelude II	CN	2	4	Full CN; neck like ‘Prelude’.
Dixie	CN ^w	2	4	looks like semi-CN; only slight crook, especially in smaller fruit; medium thick neck.
Destiny III	CN ^w	2.5	5	More full crooks than ‘Dixie’ but ≈50% fruit only semi-crook; medium thick neck.
Pavo	CN ^w	2.5	5	≈50% of fruit semi-crooks; medium thick neck.
Goldie	sCN	3	4	Medium thick neck; skin slightly rough (possibly from virus).
Picasso	sCN	1.5	3	Pale to greenish; neck longer and thinner than other semi-crooknecks.
Dividend	Z	3	5	Nearly perfectly cylindrical; very nice looking.
Revenue	Z	3	5	Nearly perfectly cylindrical; very nice.
Spineless Beauty ^v	Z	3	4	Mostly cylindrical w/ slightly larger diameter blossom end; unexposed side slightly lighter color than ‘Dividend’ and ‘Revenue’; most fruit slightly curved.
Jaguar	Z	4	2	Cylindrical; often thin with many “nubs” and misshapes.
Tigress ^v	Z	4	5	Nearly cylindrical; ≈10% of fruit slightly curved; fruit slightly larger diameter at blossom end.
Zucchini Elite	Z	3.5	4	Longer than ‘Dividend’ & ‘Revenue’; ≈10% of fruit slightly curved; slightly larger diameter at blossom end.
President	Z	3.5	3	Longer than ‘Dividend’ & ‘Revenue’; ≈50% of fruit slightly curved/crescent shaped; noticeably yellowed on unexposed side on some fruit.
Senator	Z	3.2	4	Similar to ‘Zucchini Elite’; slightly curved near stem end of most fruit.
Independence II (XPHT 1776)	Z	3	4	Similar to ‘Senator’, ‘Zucchini Elite’; fruit slightly curved near stem end in some fruit.
Declaration II (XPHT 1814)	Z	3.5	3	Similar to ‘Senator’ and ‘Zucchini Elite’; ≈40%–50% of fruit curved to “C”-shaped (may affect marketability).
XPHT 1777	Z	4	3.5	Similar to ‘Senator’, ‘Zucchini Elite’; nice dark color but may accentuate yellowing on unexposed side; ≈10% “C”-shaped.

^zCompany descriptions: SN = straightneck, SN-Py = straightneck with precocious yellow gene, CN = crookneck, sCN = semicrookneck.

^yColor ratings for yellow squash: 1 = pale yellow with greenish tint; 5 = bright golden yellow; for zucchini, 1 = lightest green; 5 = nearly black.

^xAppearance ratings: 1 = worst; 5 = best taking into account, in order of importance, shape, shape uniformity, color, and defects.

^wAlthough described as crooknecks, these cultivars did not exhibit pronounced crooks.

^vFruit from one observation plot.

ventional cultivars although this difference was not statistically significant. Again, mostly mild symptoms were observed in the transgenic plants and these probably had no impact on yields.

POWDERY AND DOWNY MILDEW. Powdery mildew symptoms were first observed inside leaf canopies of some cultivars on 15 Sept. Percentages of upper leaf surfaces exhibiting powdery mildew symptoms after the final harvest were significantly less for 'Prelude' and 'Prelude II' than for 21 out of the 23 other squash entries in the trial (Table 1). Other entries with high to moderate powdery mildew tolerance were 'Independence II', 'Senator', XPHT 1816, 'Sundance', and 'Picasso' (Table 1). Several entries appeared quite tolerant to downy mildew infections. Leaf surface area with symptoms was 10% or less for XPHT 1777, 'Zucchini Elite', 'Independence II', and 'Fortune'. 'Declaration II', 'Multipik', 'Senator', and 'Jaguar' all showed 25% or less of their upper leaf surfaces with downy mildew symptoms.

FRUIT APPEARANCE. Entries varied in color, shape, overall appearance, and potential marketability (Table 3). 'Fortune', 'Seneca Supreme', and XPHT 1817 were considered to have the most attractive fruit among yellow straightneck entries. All of the crookneck cultivars had acceptable appearance scores with the exception of 'Picasso'. 'Dividend' and 'Revenue' were standouts among zucchini cultivars with the highest fruit appearance ratings (Table 3). Fruit of 'Tigress' (included in the trial as an observation plot) also received the highest appearance rating while 'Spineless Beauty', 'Zucchini Elite', 'Senator', and 'Independence II' had good overall appearance scores. 'Jaguar' and 'Declaration II' received low ratings for a significant number of misshapen fruit.

Extracts from transgenic cultivar leaf samples sometimes produced positive ELISA reactions to antisera for the virus(es) to which that cultivar is resistant. CMV and some of the WMV ELISA positives we have reported from transgenic lines were apparently detections of viral coat proteins produced by transgenes in the resistant cultivars

rather than from virus infections in the field. Our experience with this trial indicates that considerable care is called for in the conduct and interpretation of both visual assessments and ELISA tests for viruses in field trials that include coat protein-mediated resistant materials.

Results from this fall-harvested trial together with those from similar trials in other states indicate that squash cultivars transgenic for viral coat proteins can provide excellent resistance to two or more of the viruses involved in mixed infections in the southeastern United States. Marketable yields in late plantings can be expected to vary considerably among cultivars from year to year and location to location depending on the resistance in the cultivar, the virus mix in the field, and the growth stage at which the crop is infected. Precocious yellow straightneck cultivars remain a good choice for high yields and masking of green fruit symptoms associated with moderate epidemics of WMV and CMV. Transgenic virus-resistant yellow crooknecks far outperformed popular conventional hybrids under the WMV epidemic conditions in this trial. New cultivars with gene constructs combining coat protein-mediated resistance to WMV, ZYMV, CMV, and PRSV would appear to be the logical choice for fall-harvested crookneck squash in the southeast. Both transgenic resistant zucchini breeding lines and cultivars with virus tolerance from conventional breeding were high yielding under moderately severe WMV epidemic conditions. Final cultivar choices are likely to depend more on horticultural and market characteristics when virus resistance or tolerance is adequate.

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