Effect of Zucchini Yellow Mosaic Virus on Development and Yield of Cantaloupe (*Cucumis melo*)

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ABSTRACT

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Zucchini yellow mosaic virus (ZYMV) causes a severe disease of cucurbit crops that incites stunting, chlorosis, deformation, and flower reduction, all resulting in yield loss. In a randomized complete block design, we documented the effect of ZYMV on cantaloupe (*Cucumis melo*) and showed that inoculation with this virus during vegetative and early flowering stages caused 94 and 76% reduction of marketable fruit, respectively. Plants inoculated after fruit set and healthy control plants produced a comparable number of marketable fruit. Fruit quality, as indicated by fruit size and percentage of soluble solids, was reduced by ZYMV infection during vegetative and early flowering stages but not when plants were infected after fruit set.

Zucchini yellow mosaic virus (ZYMV) causes a viral disease that recently has become established in cultivated cucurbits throughout the world. Substantial economic losses have been associated with ZYMV in cantaloupe (*Cucumis melo* L.) and other melons (13,14). Nameth et al (12) reported that: "ZYMV is probably the greatest single threat to the cucurbit industry in California." It was found first in Italy and France in 1981 and subsequently in Spain, Germany, Morocco, Israel, Lebanon, and the United States (7,9,10,17). Although a few members of other plant families serve as hosts, almost all members of the Cucurbitaceae that have been tested show symptoms of systemic infection, including severe stunting, chlorosis, and leaf and fruit deformation (7,10).

ZYMV is a potyvirus that causes symptoms resembling those incited by papaya ringspot virus (PRSV) (formerly watermelon mosaic virus-1) (19) and is related serologically to watermelon mosaic virus-2 (WMV-2) (10,18). These three viruses are transmitted by aphids in a nonpersistent manner (1,13). Several aphid species have been shown to transmit ZYMV in the laboratory, including *Myzus persicae* (Sulzer), *Aphis gossypii* Glover, *A. citricola* van der Goot, *Macrosiphum euphorbiae* (Thomas), *Acyrthosiphon pisum* (Harris), and *A. kondoi* Shinji (8,10,11,18; S. Castle, *unpublished*).

At present there are no known economically feasible means of controlling ZYMV in cucurbit crops. No commercial ZYMV-resistant cucurbit cultivars are available (16), and attempts to reduce disease incidence through vector control have been unsuccessful. Because yield reduction caused by virus infection may depend on the developmental stage of the plants, crop protection strategies that delay infection would be a useful means of maintaining a profitable yield. The focus of our study was to document foliar symptoms of cantaloupe infected with ZYMV and to determine yield reduction as a function of cantaloupe growth stage at the time of mechanical inoculation with ZYMV.

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MATERIALS AND METHODS

On 1 July 1986 at the University of California, Riverside, four rows of seeds of the cantaloupe cultivar Top Mark were planted in the field 0.9 m apart in five enclosures measuring 6.8 × 3.4 × 1.8 m high. The tops and sides of the enclosures were covered with an aphid-proof Agryl canopy cover (International Paper Co., New York, NY) to exclude viruliferous and other plant-feeding insects prevalent in the test area while allowing approximately 80% of the incident sunlight to penetrate. After 2 wk, plants were thinned to 40 per enclosure, for a density of 1.8 plants per square meter, which is similar to commercial field density. Before being planted, the experimental area was fertilized with 336 kg/ha of N and 420 kg/ha of P; then, 11 kg/ha of N was applied at 2-wk intervals until harvest. Plants were drip-irrigated once a week throughout the experiment. Because the enclosures excluded bees, which normally are required to pollinate melons, plants were hand-pollinated daily with pollen from uninoculated, symptomless plants.

A randomized complete block design was used so that each of the five enclosures represented a block. Plots were created by dividing the plants into four groups of 10 with polyethylene barriers to prevent virus infection of uninoculated controls by contact. Plants were inoculated with ZYMV at three growth stages: treatment 1 on 30 July, a vegetative stage when most plants had four true leaves; treatment 2 on 20 August, an early reproductive stage when most plants had produced one perfect flower; and treatment 3 on 8 September, a late reproductive stage when most plants had set fruit. An uninoculated control served as treatment 4.

A 1985 Imperial County (California) isolate of ZYMV was maintained in *Ranunculus sardous* Crantz to ensure isolation from other cucurbit viruses found in that area before aphid transmission to zucchini as a propagation host (11). Inoculum was obtained by grinding infected zucchini leaves in a high-speed blender with water and filtering the extract through cloth. The extract was mixed with a small amount of Carborundum to facilitate abrasion of the leaf epidermis. This preparation was applied with an airbrush at the aforementioned times to the oldest true leaf of each plant in treatment 1 and to the third and fourth mature leaves from the terminal in treatments 2 and 3. Successful inoculation on individual plants was confirmed by observing symptom development.

Plants were treated with triadimefon (50W, 0.10 g/L) three times to control powdery mildew, caused by *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. Oxydemetonmethyl (2 EC, 5 ml/L) was used once to control a small, isolated infestation of *A. gossypii*, and fenvalerate (2.4 EC, 1.1 ml/L) was also used once to control an infestation of *Trichoplusia ni* (Hübner).

Weekly counts of staminate and perfect flowers in all treatments were recorded until the beginning of fruit set, then discontinued so that newly set fruit would not be disturbed. This restriction did not allow us to document the effect of virus infection on flower production for treatment 3. Mature fruit (i.e., full slip) were harvested and counted on 9 October. The remainder of the fruit, regardless of maturity, was harvested 16 October because plants began to degenerate owing to inclement weather. All fruit were counted, judged marketable or not, and sorted by weight into the following fruit size classes according to accepted market practice: 23, 18, 15, and 12 fruit per standard field carton (6).

Fruit were considered unmarketable if immature, deformed, cracked, rotted with a secondary infection, or undersized (>23 fruit per carton size class). Percentage of soluble solids in marketable fruit was determined by refractometry (15).

Analysis of variance followed by Duncan's (4) multiple range test was used to detect differences among treatments in weekly counts of staminate and perfect flowers per plant and in yield data. Arc sine transformations were used before analysis of percentage of marketable fruit per plant and percentage of total fruit per plant in each size class in order to ensure homogeneity of variance.

RESULTS

Symptoms of ZYMV infection appeared between 7 and 14 days after inoculation and initially consisted of abrupt reduction in flower production followed by veinclearing of the least mature leaves. Subsequent foliar symptoms included systemic mosaic, enations, and deep serration (Fig. 1A). Infected plants grew more slowly than healthy controls and deteriorated rapidly with the onset of inclement weather just before harvest.

Within 2 wk of virus inoculation, significantly ($P < 0.05$) fewer staminate flowers were produced on plants inoculated during the vegetative stage (treatment 1) and early reproductive stage (treatment 2) than on the healthy controls (treatment 4) (Fig. 2A). Production of staminate flowers on plants in treatment 1 decreased from an average of 5.6 to 1.0 flower per plant after 7 days, at which time healthy controls had an average of 10.9 flowers per plant. Plants in treatment 2 also showed a reduction in staminate flowers, with a decline from an average of 12.5 to 2.5 flowers per plant 7 days after inoculation. At this time, healthy controls had a mean of 12.0 flowers per plant.

Perfect flower production also declined after inoculation. A statistical difference in the number of perfect flowers per plant was observed only on the last sampling date, because the number of perfect flowers within any treatment was small and highly variable (Fig. 2B). After an initial decrease in perfect flower production, some ZYMV-infected plants generated a cluster of perfect flowers on the terminal bud (Fig. 1B), which did not set fruit after pollination. This was in contrast with healthy plants, on which perfect flowers were borne singly and more than four nodes proximal to the terminal bud. Production of both staminate and perfect flowers declined naturally in treatments 3 and 4 as the plants began to set fruit.

Fruit that developed after inoculation became nubby and misshapen and did not develop the netting typical of fruit from healthy plants (Fig. 1C). In addition, infected fruit commonly cracked open, allowing entry of secondary pathogens.

ZYMV infection reduced several yield parameters. The number of marketable fruit per plant, the total number of fruit per plant, and the percentages of marketable fruit per plant were significantly ($P < 0.05$) lower on plants in treatments 1 and 2 than in treatments 3 and 4 (Table 1). Compared with the healthy controls, plants in treatment 1 showed a 64% reduction in total fruit and a 98% reduction in marketable fruit. Most of the fruit from treatment 1 were undersized, and approximately one-third were rotted from a secondary infection. Only 6% of the fruit from the healthy control group had an infection caused by opportunistic fruit rotting agents. Compared with the healthy controls, plants in treatment 2 showed a 53% reduction in total fruit and a 89% reduction in marketable fruit, and plants in treatment 3 showed an 18% reduction in total fruit and a 22% reduction in marketable fruit. Yield parameters for treatments 3 and 4 were statistically similar, indicating that marketable yield was not significantly ($P < 0.05$) reduced on plants that set fruit before inoculation with ZYMV.

A significant ($P < 0.05$) difference in the size distribution of fruit harvested was observed among treatments (Table 2). In treatment 1, 90% of the fruit produced was in the undersized class (>23 fruit per carton), compared with 58% in treatment 2, 40% in treatment 3, and 44% in the healthy controls. The undersized fruit in treatment 1 were mature, whereas the undersized fruit of

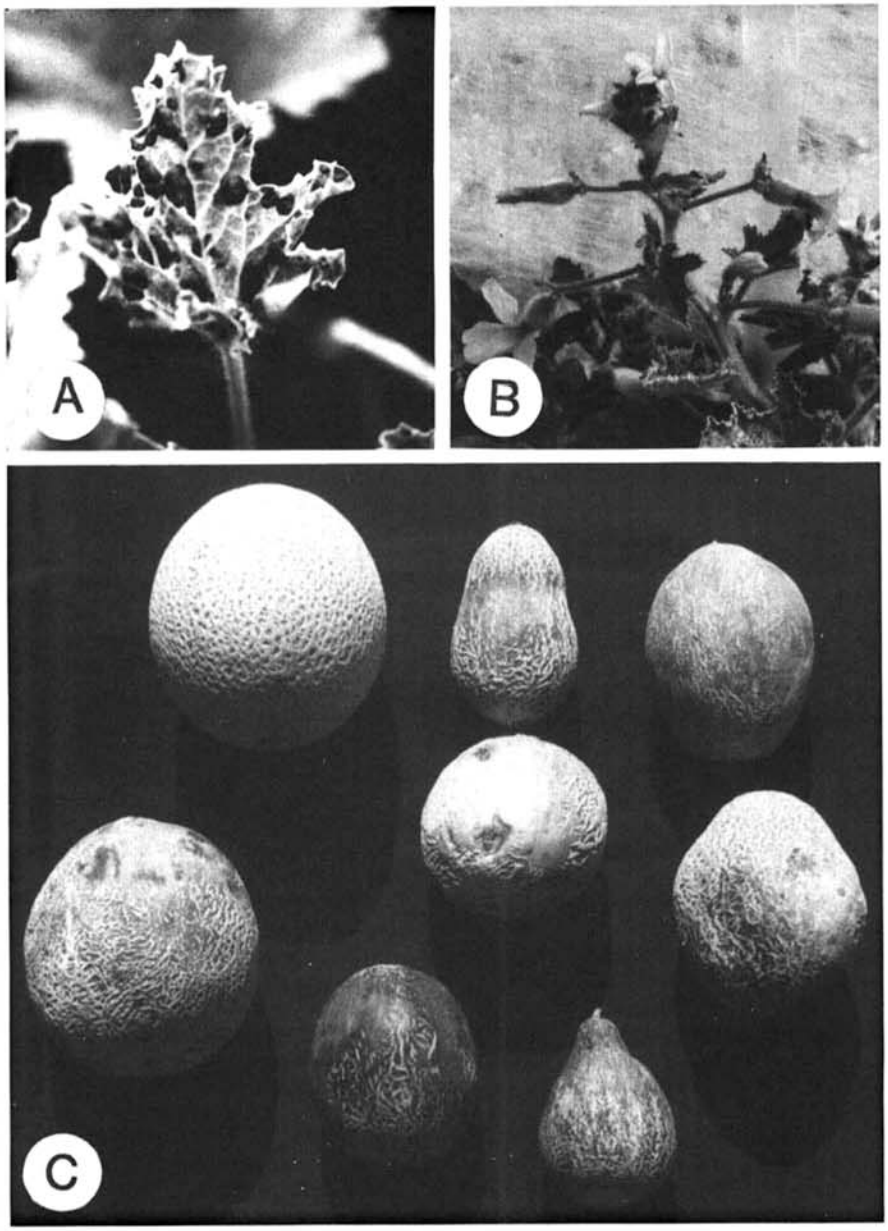

Fig. 1. Symptoms of zucchini yellow mosaic virus infection on **(A)** leaves, **(B)** flower buds, and **(C)** fruit (uninfected fruit, upper left) of field-inoculated cantaloupe.

the healthy controls were immature, indicating that most of the fruit on plants inoculated during the vegetative stage did not develop to full size. The maturation of undersized fruit in treatments 2 and 3 was variable. All treatments had a similar proportion of fruit distributed in the smallest two marketable size classes (23 and 18). The proportion of fruit in the largest two size classes (15 and 12) showed the greatest disparity with respect to treatments. Treatments 1 and 2 (inoculation before fruit set) produced a significantly ($P<0.05$) lower proportion of fruit in these size classes than did treatments 3 (inoculation after fruit set) and 4 (no inoculation). There was a trend for fruit to be larger as inoculation was delayed (Table 2).

Sugar concentration of marketable fruit, as determined by percentage of soluble solids, was significantly ($P<0.05$) lower in plants inoculated with ZYMV before fruit set than in healthy controls (Table 1). Values ranged from a mean percent soluble solids of 9.9 in fruit from treatment 2 to a mean of 11.4 in fruit from the healthy controls. The percent soluble solids in fruit from treatment 3 averaged 10.5, which was not significantly different from that in either treatment 2 or the healthy controls. Plants from treatment 1 did not provide a statistically valid sample for analysis because of the low number of marketable fruit produced.

DISCUSSION

Disease management might be facilitated by knowledge of the time period during which protection is needed. In this study, we demonstrated that after fruit set, ZYMV infection did not significantly reduce marketable yield. Thus, ZYMV need not be eradicated to achieve a profitable harvest, and our results suggest it could be managed by ecologically sound agronomic practices designed to delay the onset of disease.

Studies with other cucurbit crop and virus systems relate similar conclusions. Early inoculation of zucchini (*Cucurbita pepo* L.) with watermelon mosaic virus (WMV) resulted in a lower production of female flowers and fruit than did late inoculation (2). Similarly, inoculation of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) with WMV during the vegetative stage caused greater yield losses than inoculation after fruit set (3). Inoculation of cucumber (*Cucumis sativus* L.) with cucumber green mottle mosaic virus at the cotyledon stage caused significant yield reductions, whereas later infection had little effect on yield (5).

Several crop protection strategies, including canopy covers, intercropping, and reflective mulches, have shown the potential to retard viral diseases in cantaloupe. Use of canopy covers that excluded aphid vectors delayed transmission of ZYMV to cantaloupe by more than 2 wk (14). Because canopy covers must be removed at flowering to allow pollination, however, their effectiveness may be reduced during years in which there is heavy ZYMV and vector pressure. In field studies, Toba et al (20) showed that wheat, as a protection crop for cantaloupe, significantly delayed the onset of WMV-2 by interfering with transmission; but the wheat crop also slowed cantaloupe growth and suppressed yield. In a similar experiment, Perring (14) was unable to show a delay in onset of ZYMV infection when cantaloupe was intercropped with wheat. Recent studies have shown that the use of a silver reflective mulch reduced vector numbers and preserved yield by delaying the onset of ZYMV infection for 2 wk (14).

In order to provide better management for cantaloupe during ZYMV epidemics, future studies should focus on delaying infection until after fruit set. Contributing to these strategies would be the ability to predict heavy outbreak seasons. Since virus-delaying techniques increase production costs, this predictability would enable the grower to make better cost-effective decisions.

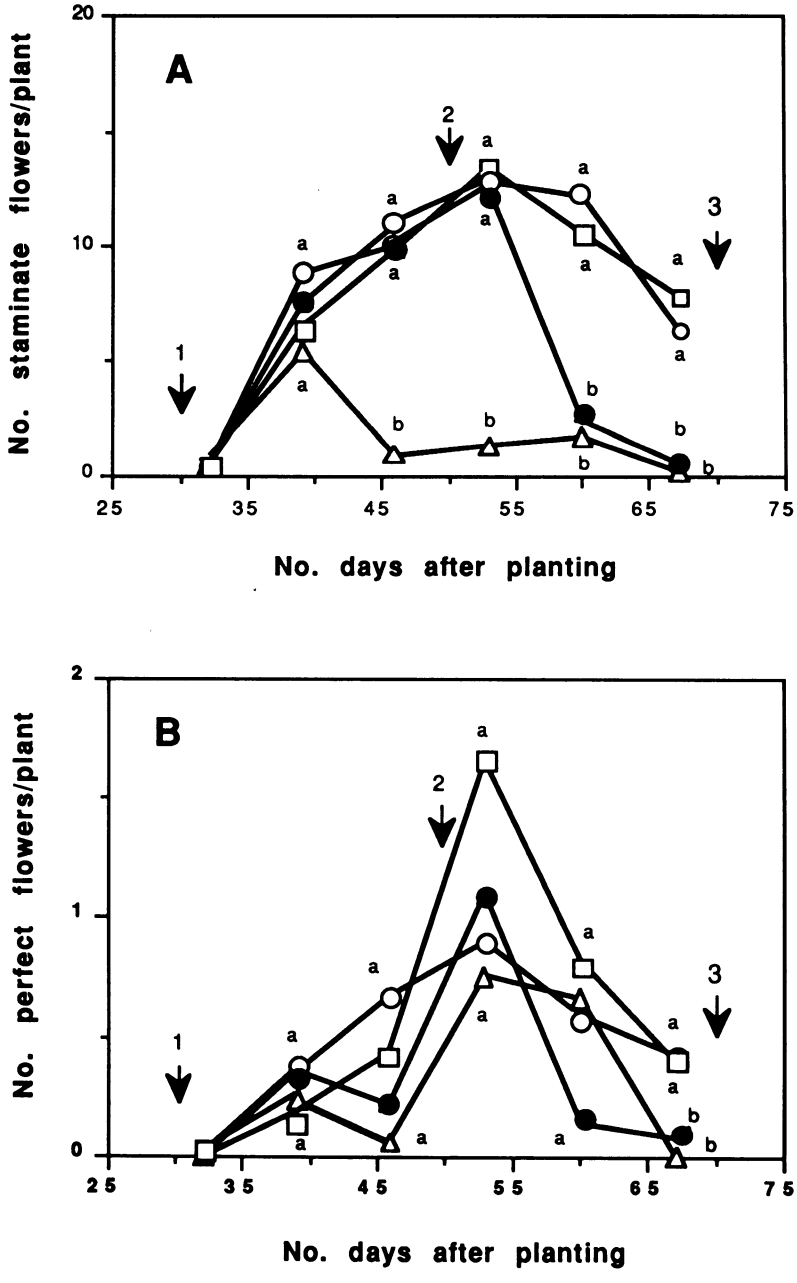

Fig. 2. Mean number of **(A)** staminate and **(B)** perfect flowers for each treatment until fruit set in cantaloupe cultivar Top Mark mechanically inoculated in the field with zucchini yellow mosaic virus. Plants were inoculated at three different growth stages (arrows): treatment 1 = vegetative (△), treatment 2 = early reproductive (•), and treatment 3 = late reproductive (o); treatment 4 = uninoculated control (□). Means with the same letter on the same day are not significantly different ($P<0.05$).

Table 1. Mean total numbers of fruit per plant and of marketable fruit per plant and percentages of total marketable fruit per plant and of soluble solids from fruit for each treatment produced by cantaloupe cultivar Top Mark mechanically inoculated in the field with zucchini yellow mosaic virus

Treatment no.	Growth stage at inoculation	Total no. fruit/plant	No. marketable fruit/plant	Percentage of marketable fruit/plant	Percentage of soluble solids
1	Vegetative	0.9 b[y]	0.0 b	3.2 b	...[z]
2	First perfect flower	1.2 b	0.2 b	12.2 b	9.9 b
3	First fruit set	2.2 a	1.0 a	47.4 a	10.5 ab
4	Healthy control	2.6 a	1.3 a	49.8 a	11.4 a

[y] Values followed by the same letter within a column are not significantly different ($P < 0.05$).
[z] Statistical sample not available because of low production of marketable fruit.

Table 2. Percentage of total number of fruit per plant in each of five size classes (number per carton)[y] for each treatment produced by cantaloupe cultivar Top Mark mechanically inoculated in the field with zucchini yellow mosaic virus

Treatment no.	Growth stage at inoculation	Percentage of total no. fruit/plant in each size class				
		<23	23	18	15	12
1	Vegetative	90.0 a[z]	4.5 a	3.1 a	0.0 b	2.3 b
2	First perfect flower	58.7 b	10.6 a	21.3 a	5.7 b	3.6 b
3	First fruit set	40.6 b	12.8 a	20.6 a	17.7 a	8.3 ab
4	Healthy control	44.8 b	9.8 a	14.8 a	15.9 a	14.5 a

[y] Johnson and Mayberry (6).
[z] Values followed by the same letter within a column are not significantly different ($P < 0.05$).

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