

Occurrence of Mosaic Viruses in Melons in the Central Valley of California

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ABSTRACT

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Three melon-growing regions in the Central Valley of California were surveyed for the incidence and severity of watermelon mosaic virus 2 (WMV2), zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), and papaya ringspot virus–watermelon strain (PRSV-W), during 1988 and 1989. WMV2 was the most prevalent virus in all three regions (Yolo/Sutter/Yuba counties, Stanislaus County, and Merced/Fresno counties), both in the number of sites with infected plants and in the proportion of symptomatic plants within each site. CMV and ZYMV were detected in fewer sites and generally infected 20% or fewer symptomatic plants within a site. In Stanislaus County, ZYMV, which had not previously been reported in the Central Valley, was detected in a higher number of sites and a higher proportion of plants per site in 1989 than in 1988. The increased incidence of ZYMV is of concern because this virus is severely pathogenic. PRSV-W was detected in low levels in the growing areas of Stanislaus and Merced/Fresno counties. This study suggests that management strategies for dealing with virus diseases in the agriculturally diverse Central Valley will be specific to each particular region.

Mosaic diseases caused by arthropod-borne viruses are a worldwide problem in cucurbits and have become an increasing problem in melons (*Cucumis melo* L.), including honeydew, cantaloupe, and specialty melons (such as crenshaw and casaba), which account for the majority of cucurbits grown in the Central Valley of California (19). During 1988 and 1989, the melon crop values were estimated to be \$242 million and \$252 million, respectively (4). Four aphid-borne viruses known to occur in California include watermelon mosaic potyvirus 2 (WMV2), papaya ringspot potyvirus–watermelon strain (PRSV-W), zucchini yellow mosaic potyvirus (ZYMV), and cucumber mosaic cucumovirus (CMV) (19). These viruses are transmitted in a stylet-borne, nonpersistent manner by aphids (24), and disease symptoms include systemic chlorotic mottling or

speckling of leaves, vein clearing, deformed leaves, and stunted and deformed fruit. If the plant is infected early in its growth, before fruit is formed, fewer flowers may be produced and the fruit may be aborted, causing partial or entire loss of the crop (2,3,7). In the case of ZYMV and PRSV-W, if the infection occurs later in the growth of the plant, the fruit may develop distortions and spots that render it unmarketable. In either case, damage and yield losses can be extensive (19,22).

The distribution, severity, and relative proportions of the four viruses can change from year to year. Surveys of melons in the deserts of southern California and Arizona conducted from the 1950s through 1981 demonstrated that WMV2 and CMV were the most prevalent viruses (8,11,16,20,34). In 1983, ZYMV was detected for the first time in the Imperial Valley of California (18,19). Recent studies indicated that ZYMV and WMV2 are the dominant viruses in this southernmost region of California (22), and their temporal and spatial distributions vary with vector pressure, weather, and proximity to virus sources.

Although extensive work had been conducted on melons in southern California, little research had been done in the geographically distinct and agriculturally diverse Central Valley. A survey of the Cen-

tral Valley was conducted in order to develop virus management strategies for this region. The specific objective of the survey was to document the distribution and abundance of WMV2, ZYMV, CMV, and PRSV-W in melons in the three major melon-growing regions of the Central Valley: Yolo/Sutter/Yuba counties, Stanislaus County, and the Merced/Fresno counties.

MATERIALS AND METHODS

Surveys for infected melon crops. Honeydew, cantaloupe, and specialty melon fields (including casaba and crenshaw) were surveyed for mosaic viruses during July and August 1988 and August and September 1989 in three major growing regions of Central California separated by 40 to 70 miles. Melons were planted from late April until early July in the Central Valley region. During the 1988 and 1989 field surveys, respectively, 95.4 and 67.6% of Yolo/Sutter/Yuba fields, 70.0 and 83.3% of Stanislaus fields, and 76.0 and 55.1% of Merced/Fresno fields had fruit larger than 5.1 cm (2 in) in diameter. Thus, the majority of the fields were in the late stage of plant development during the survey.

The percentage of mosaic virus in each field was estimated visually by examining 100 melon plants in the northwest and the southeast corners of each field. Because the prevailing wind in the Valley is from the northwest, it was hoped that this sampling scheme would give us information about the epidemiologies of the viruses. The terminal three leaves of up to 10 melon plants exhibiting mosaic virus symptoms were collected from each corner. Each plant sample was kept separately in a plastic bag in a freezer (–20°C). The growth stage of the plants was recorded, and surrounding fields (crops, weeds, disked fields, and barriers such as buildings, trees, and other possible obstructions to aphid movement) were described for each melon field surveyed.

Identification of virus isolates. Plant samples were tested for the presence of CMV, ZYMV, PRSV-W, and WMV2 using indirect enzyme-linked immunosorbent assay (ELISA) (9). ZYMV and PRSV-W antisera were provided by D. E. Purcifull (University of Florida, Gainesville), and

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CMV and WMV2 antisera were provided by J. A. Dodds (University of California, Riverside). For each plant sample, 0.5 g of tissue was weighed, leaf sap was expressed using a revolving plant crusher (Model 1, Ravenel Specialties Company, Seneca, SC), and sap was diluted 1:1,000 (wt/vol) in coating buffer (1.59 g of Na₂CO₃, 2.93 g of NaHCO₃, and 0.2 g of NaN₃ in 1 liter of double-distilled H₂O, adjusted to pH 9.6) and incubated for 1.5 h at 35°C in flat-bottom Immulon I microtiter plates (Dynatech, Alexandria, VA). For positive controls, three wells of each microtiter plate contained tissue extracts from summer squash (*Cucurbita pepo* L.) plants mechanically inoculated with one of the four viruses (5). Negative controls consisted of three wells containing nonsymptomatic, field-collected honeydew melon tissue extracts. Virus IgG (1:10,000 dilution) was added to the wells and incubated for 1.5 h at 35°C. Goat anti-rabbit IgG (1:6,000 dilution) conjugated with alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added to the wells and incubated for 1.5 h at 35°C. Substrate (Sigma 104) was added to the wells and incubated for 0.5 h at 35°C. Optical densities (OD) of each sample were determined using a Vmax microtiter plate reader (Molecular Devices Co., Menlo Park, CA) at 405 nm. A sample was considered virus-positive if the OD exceeded the mean plus three standard deviations of the OD of the healthy tissue controls.

RESULTS AND DISCUSSION

Yolo/Sutter/Yuba region. In Yolo/Sutter/Yuba counties, the northernmost area, 44 and 34 honeydew fields were sampled during July and August 1988 and August and September 1989, respectively (Table 1). These numbers represent more than 90% of the melon fields planted in this area during those years. Of those sampled fields, 52% in 1988 and 56% in 1989 had melon plants that exhibited symptoms and tested positive for one or more of four viruses. The most widespread virus detected was WMV2 (48 and 56% of sites visited in 1988 and 1989, respectively). CMV also was detected in 25 and 12% of the sites visited during those years. ZYMV was detected only in 9% of the 1988 field

sites, and PRSV-W was not detected in any plant samples tested. The percentage of sites with more than 33% total mosaic symptoms in either the NW or SE corner of the field was 14 and 15% in 1988 and 1989, respectively (Table 1).

In addition to a higher number of fields, WMV2 was also detected in a higher proportion of symptomatic melon plants within the sampled area of each field. For example, in 1988 (Fig. 1), there were 11 fields in which more than one virus was

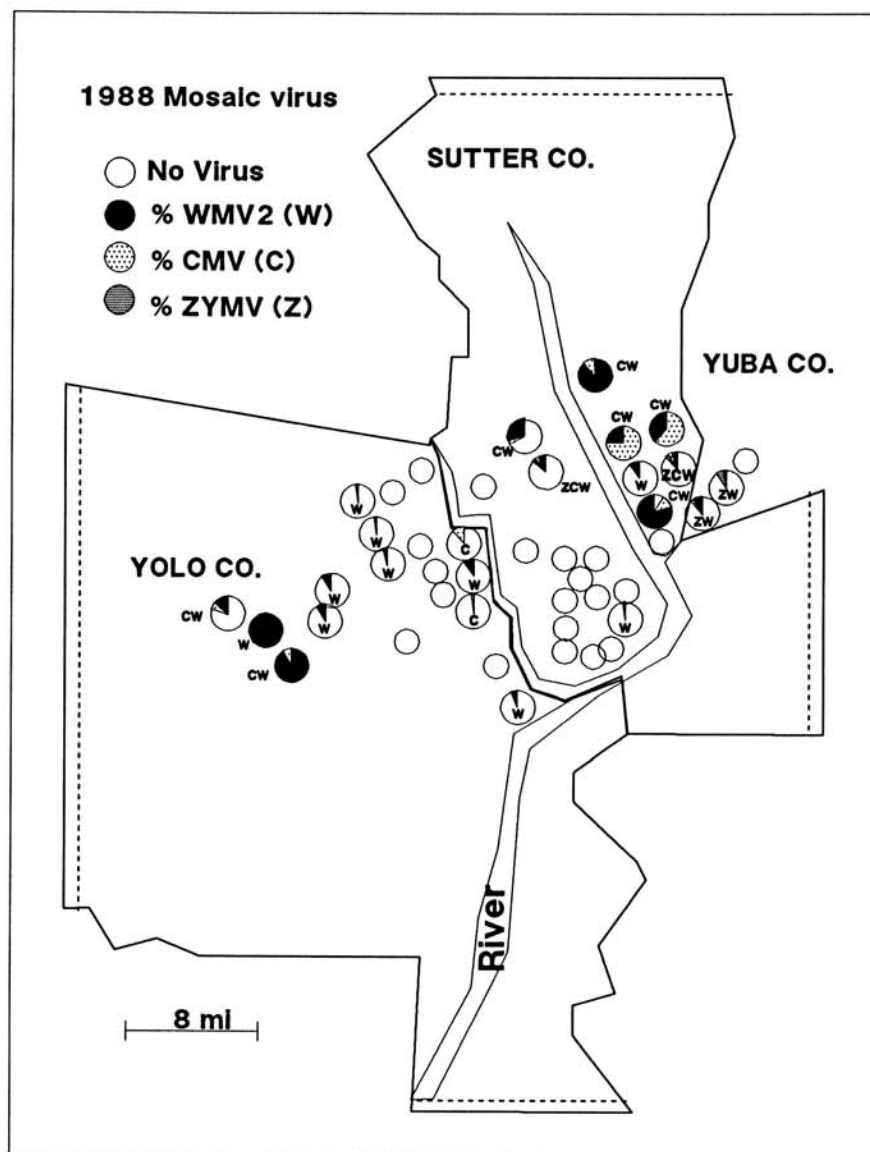


Fig. 1. The distribution of melon fields and the incidence of mosaic symptoms (single infections of watermelon mosaic virus 2 [WMV2], cucumber mosaic virus [CMV], and zucchini yellow mosaic virus [ZYMV]) during 1988 in the Yolo/Sutter/Yuba region. The proportions of each virus are from the most heavily infected NW or SE corner of each field.

Table 1. Results of mosaic virus survey of melon fields during the 1988 and 1989 field season in the Central Valley of California

Growing region Collection date	Fields sampled (no.)	Fields infected (%)	Sampled fields with mosaic virus (%)				Melon fields with >33% total virus infection in NW or SE corner (%)
			WMV2	CMV	ZYMV	PRSV-W	
Yolo/Sutter/Yuba							
Jul-Aug 1988	44	52	48	25	9	0	14
Aug-Sep 1989	34	56	56	12	0	0	15
Stanislaus							
Aug 1988	40	75	70	25	3	8	35
Aug 1989	30	90	87	20	30	0	53
Merced/Fresno							
Jul-Aug 1988	92	16	7	9	5	1	1
Aug 1989	107	28	23	11	5	0	3
Sep 1989	8	100	100	63	71	14	75

detected, and in eight of these fields WMV2 was detected in a higher proportion of the symptomatic plants. In 1989 (Fig. 2), WMV2 was detected in a higher proportion of symptomatic plants sampled in all four fields where CMV was also detected. ZYMV was detected in less than 5% of the leaf samples from any field.

Mixed infections of virus in the same leaf sample were relatively rare. In 1988 and 1989, respectively, we detected more than one virus in 6.1 and 4.4% of the leaf samples showing symptoms of infection. These mixed infections consisted solely of WMV2 + CMV.

WMV2 was detected in fields distributed throughout the Yolo/Sutter/Yuba growing region in both 1988 and 1989 (Figs. 1 and 2). CMV was detected in 11 melon fields found throughout the growing region during 1988 but only in four fields in the northeastern corner of the growing region during 1989. ZYMV was detected

only in the northeastern area of the growing region during 1988, so the threat of this virus was relatively small and isolated.

Neighboring crops of the Yolo/Sutter/Yuba melon fields included alfalfa (2.1%), beans (3.9%), carrots (0.4%), corn (1.6%), rice (34.6%), safflower (11.0%), stone fruits (5.5%), sugar beets (5.1%), sunflower (3.9%), tomatoes (18.1%), walnuts (5.9%), and wheat (7.9%). WMV2 and CMV have a wide plant host range including beans, sugar beets, and weeds for WMV2 (12,20,22) and beans, carrots, corn, safflower, sugar beets, sunflower, tomatoes, and weeds for CMV (20,22,25). Thus, the neighboring crops and their associated weeds could have served as virus and aphid reservoirs (22).

Disease incidence was high during 1988 (Fig. 1) and 1989 (Fig. 2) in melon fields in the northeastern and southwestern edges of the Yolo/Sutter/Yuba growing region. In contrast, in the central area of the three-

county growing region, the disease incidence was low in both years. In the central area, 14 of 15 melon fields in 1988 and 17 of 17 melon fields in 1989 had an average of 2.3 borders of rice as the neighboring crop. Rice is a relatively weed-free crop and does not act as a host of these viruses, nor is it a source of aphid species that vector these viruses. The outer growing areas, experiencing higher disease incidence, had only six of 29 melon fields in 1988 and three of 17 melon fields in 1989 with rice as a neighboring crop. These data suggest that absence of weeds and the presence of the rice crop that is not a source of virus or aphids kept the incidence of virus low in the central area of the Yolo/Sutter/Yuba melon-growing region.

Stanislaus region. In Stanislaus County, 40 and 30 fields were surveyed during August of 1988 and 1989, respectively (Table 1). There were seven and 15 specialty melon fields, 28 and 15 honeydew fields, and five and three cantaloupe fields in 1988 and 1989, respectively. These fields represented more than 85% of fields planted with melons in those years. Of those surveyed fields, 75 and 90% tested positive for one or more of the viruses. Similar to the Yolo/Sutter/Yuba growing region, the most prevalent virus was WMV2: 70 and 87% of the 1988 and 1989 sites surveyed. CMV was present in 25 and 20% of the sites and ZYMV in 3 and 30% of the sites for 1988 and 1989, respectively, and PRSV-W was only present in 8% of the 1988 sites. The percentages of sites with more than 33% total disease symptoms in either the NW or SE corner of the field were 35 and 53% in 1988 and 1989, respectively (Table 1). Thus, the percentage of heavily infected fields was higher for Stanislaus County than for the Yolo/Sutter/Yuba region. The differences between the two counties appeared to be in the percentage of sites with WMV2- and ZYMV-infected plants. The increase of ZYMV infection from 3% in 1988 to 30% in 1989 in Stanislaus County might indicate that this virus is spreading. Alternatively, ZYMV has a more limited host range (22), and so disease incidence may vary from year to year because of variation in overwintering survival of its host.

In addition to infecting the highest percentage of Stanislaus County melon fields, WMV2 was detected in a higher proportion of symptomatic melon plants within the sampled area of each field. In 1988 (Fig. 3), WMV2 was detected in the highest proportion of symptomatic plants sampled in eight of nine melon fields with more than one virus infection. In 1989 (Fig. 4), WMV2 was detected in a higher proportion of sampled plants in eight of 12 melon fields with multiple infections. Proportions of ZYMV- and CMV-infected leaf samples varied from field to field and year to year. PRSV-W was never detected in greater than 10% of the leaf samples from any field.

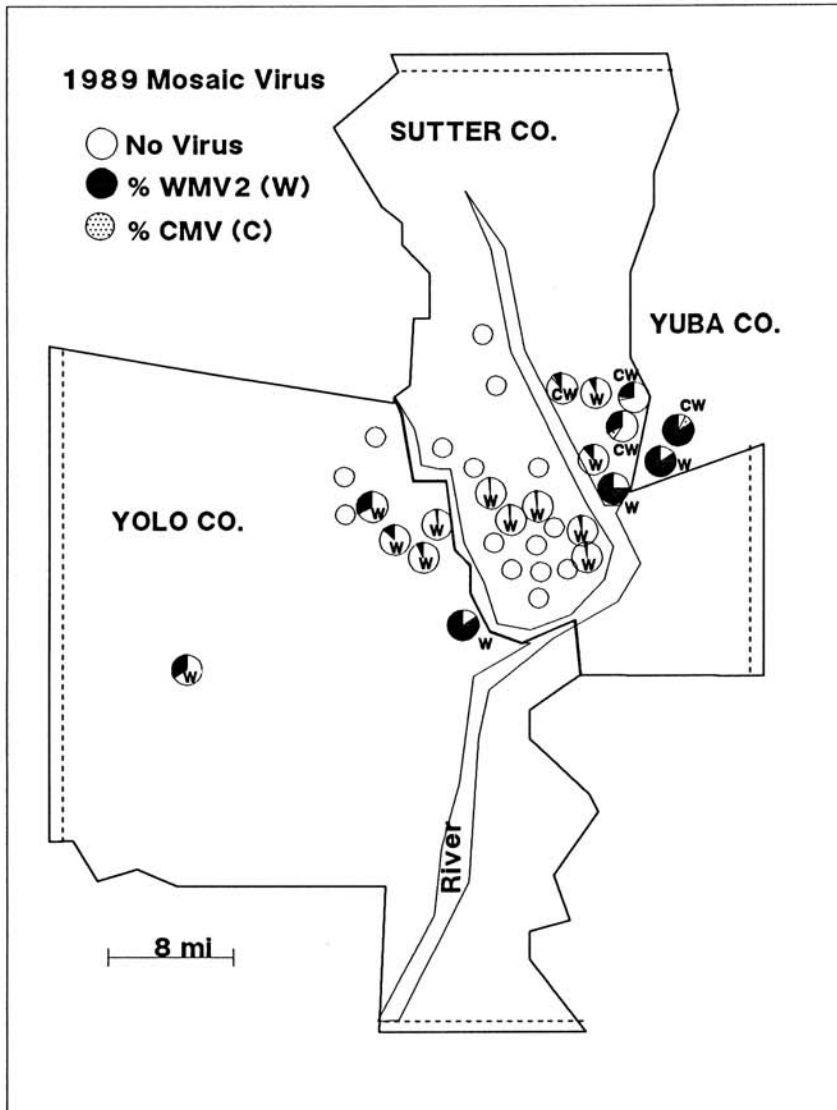


Fig. 2. The distribution of melon fields and the incidence of mosaic symptoms (single infections of watermelon mosaic virus 2 [WMV2] and cucumber mosaic virus [CMV]) during 1989 in the Yolo/Sutter/Yuba region. The proportions of each virus are from the most heavily infected NW or SE corner of each field.

As in the Yolo/Sutter/Yuba region, mixed infections of virus in the same plant sample were relatively rare in Stanislaus County. In 1988 and 1989, respectively, we detected both WMV2 and CMV in 2.7 and 2.0% of the leaf samples showing symptoms of infection. We detected both WMV2 and PRSV in 0.8% of the leaf samples showing symptoms in 1988. In 1989, we detected mixed infections of WMV2 and ZYMV in 6.7% of the leaf samples tested.

In contrast to the Yolo/Sutter/Yuba growing region, the entire growing region of Stanislaus County experienced relatively high disease incidence. WMV2 and CMV were detected in fields throughout the Stanislaus growing region in both 1988 and 1989 (Figs. 3 and 4). ZYMV was detected in only one field in the central area of the growing region in 1988 and in nine fields in the northern, central, and southern areas of the growing region in 1989. This increase in the number of fields infected with ZYMV and the proportion of ZYMV-infected plants within those fields suggests that ZYMV increased in severity in central Stanislaus County. This should be of concern to melon growers, since this is a highly aggressive and damaging disease. PRSV-W was detected in only three fields located in northern or southern areas of this region during 1988.

The Stanislaus melon fields were located next to alfalfa (8.2%), beans (22.8%), carrots (0.4%), corn (0.4%), crucifers (2.3%), herbs (2.7%), nuts (27.5%), onions (0.4%), peppers (1.6%), stone fruits (14.4%), tomatoes (18.5%), and wheat (0.8%). Many of these crops were also found in the Yolo/Sutter/Yuba melon-growing region. Two noticeably abundant neighboring field crops in Stanislaus were beans and tomatoes. Beans act as a host for CMV and WMV2 (22,25), and tomatoes act as a host for CMV (25). Additionally, tree crops including walnuts, pistachios, almonds, plums, apricots, cherries, and nectarines were abundant in Stanislaus County. While these crops are not known to be reservoirs of these viruses, the weeds commonly found on the orchard floor can host both viruses and aphids (22). Thus, the high number of weeds and crops that could harbor viruses and aphids is likely to have contributed to the disease epidemic in the Stanislaus area. Another factor that may have accelerated the virus epidemic in the Stanislaus growing area was the close proximity of melon fields to each other (Figs. 3 and 4). Most of the sampled fields were found in a narrow 3-mile-wide band in the central area of the county, and the fields tended to be planted in clusters. Once a virus epidemic was initiated, it was easily moved to the next field.

Merced/Fresno region. In the Merced/Fresno area, 92 and 107 fields were surveyed in July and August 1988 and August 1989, respectively (Table 1). There were

one and three specialty melon fields, three and nine honeydew fields, and 88 and 103 cantaloupe fields in 1988 and 1989, respectively. The surveyed fields represented more than 70% of the acreage that was planted with melons. Of these fields, only 16% (1988) and 28% (1989) showed any disease symptoms, and only 1 and 3% of these sites had moderate to high levels of disease. Thus, virus epidemics were not a serious concern in the majority of Merced/Fresno area melon fields during July and August 1988 and August 1989. The Merced/Fresno area is the southernmost growing region of the three studied and generally has the longest growing season.

In the Merced/Fresno growing region, where disease incidence was low, the percentage of sites with WMV2 was only

slightly higher than the percentage of sites with CMV or ZYMV in the July to August surveys (Table 1). The proportions of sampled plants within those fields that were found to be infected with WMV2 were higher compared to the other viruses in four of five melon fields during 1988 and four of 11 fields in 1989. In 1988 and 1989, WMV2 and CMV were detected in fields in many areas of this growing region. In 1988, ZYMV was detected in four fields in Fresno County, and in 1989 ZYMV was detected in four fields in the central area of the growing region. The distribution and incidence of ZYMV did not appear to be expanding between 1988 and 1989.

As in the other regions, mixed infections of virus in the same plant sample were relatively rare in the Merced/Fresno region

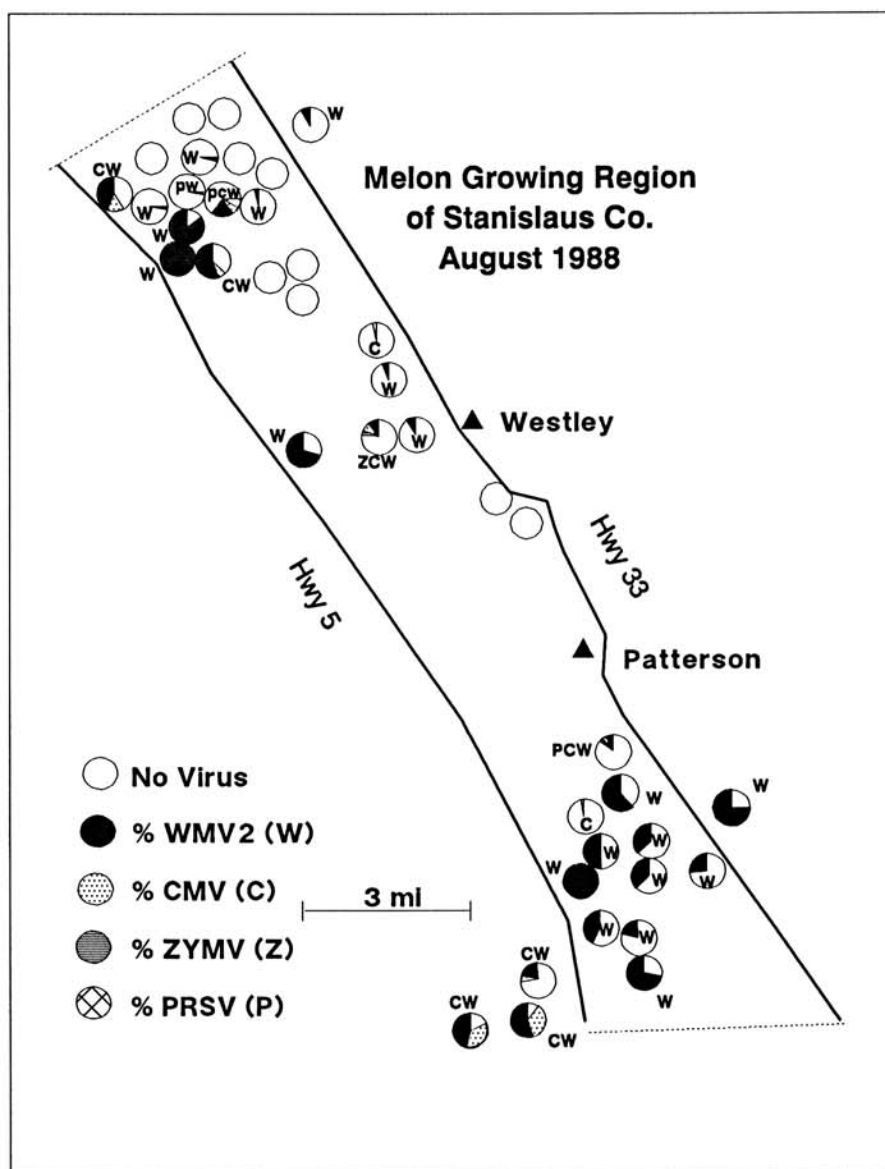


Fig. 3. The distribution of melon fields and the incidence of mosaic symptoms (single infections of watermelon mosaic virus 2 [WMV2], cucumber mosaic virus [CMV], zucchini yellow mosaic virus [ZYMV], and papaya ringspot virus–watermelon strain [PRSV-W]) during 1988 in the Stanislaus County region. The proportions of each virus are from the most heavily infected NW or SE corner of each field.

during 1988 and August 1989. We detected WMV2 and CMV in 5.5 and 2.6% of the leaf samples showing symptoms of infection. We detected WMV2 and ZYMV in 1.3% of the leaf samples showing symptoms in 1989.

In September 1989, an outbreak of mosaic symptoms occurred in late-season melon plantings, and of the eight infected sites sampled, all were infected with WMV2, five with CMV, six with ZYMV, and one with PRSV-W (Table 1). Mixed infections of ZYMV, WMV2, and CMV were common at this time of year. Of plants with disease symptoms, 15.9% were WMV2 + CMV, 17.3% WMV2 + ZYMV, 4.3% ZYMV + CMV, and 7.2% WMV2 + ZYMV + CMV. All four viruses were a problem in this region; however, damage was severe only in the small percentage of

melon fields that had been planted late in the season (end of July).

The Fresno/Merced area had many of the same crops as the other two growing regions, including alfalfa (23.3%), beans (4.3%), corn (7.5%), cotton (35.8%), nuts (2.8%), onions (0.3%), peppers (0.3%), rice (2.5%), safflower (0.3%), stone fruits (3.5%), sugar beets (9.0%), sunflower (0.6%), tomatoes (7.3%), and wheat (2.5%). Thus, there does not seem to be a lack of plant material in this region that can harbor the aphid vectors or host the viruses. The 1989 virus sampling of eight late-season melon fields demonstrated clearly that all four viruses were present in this area and could become a serious problem. This suggests that mosaic viruses are present throughout the growing region; however, they may not develop into a seri-

ous problem until late in the season. Later plantings are more likely to be infected in an early growth stage and so sustain more damage.

These surveys indicate that WMV2 is the most widely distributed and abundant virus in these three melon-growing regions of the Central Valley. CMV and ZYMV were the next most prevalent viruses, the abundance depending on location and time of year. PRSV-W was rare in the Central Valley. The dominance of WMV2 may change if ZYMV becomes more broadly distributed. This has occurred in southern California, and ZYMV rapidly has become the most damaging virus of cucurbits in that area (19,22). Since it was first described by Lisa et al. (13), ZYMV has become a problem in cucurbits in many regions of the United States (6,10,17,26,27,28,31,35) besides California. It is thought that the increasing incidence of ZYMV may be due to the more aggressive and competitive nature of ZYMV in mixed infections, displacing WMV2 in the host plant (6).

Currently, the most successful method of mosaic virus disease control centers around plant resistance to a particular pathogen. Our surveys of melons in the Central Valley suggest that breeding melons or developing transgenic plants with resistance to WMV2 would greatly reduce damage due to aphid-borne pathogens in this area. Researchers should also direct efforts toward developing resistance to ZYMV, which is heretofore limited in distribution but is highly pathogenic. Cross protection, mechanically infecting melons with mild strains to protect them from severe strains of ZYMV, may also be useful for combating this disease (21,32,33). Since ZYMV has a narrow host range relative to WMV2 (22), growers may be able to minimize disease caused by ZYMV through diligent surveys of the areas around their fields and removal of overwintering weeds and cucurbits.

Our data suggest that in some regions it may be easier to manage these aphid-borne viruses than in other regions. In the Yolo/Sutter/Yuba counties, disease incidence was highest in areas surrounded by crops and weeds known to host WMV2 and CMV, and lowest where rice was the primary neighboring crop. A virus-management strategy for this region might be to concentrate melon fields in areas where rice is the dominant crop. In Stanislaus County, disease incidence was high throughout the county during both years of our study, suggesting that this will be the most difficult region in which to control disease. Without the availability of disease-resistant cultivars and with the wide host range of WMV2 and CMV, this region will require an aggressive program of integrated tactics that repel aphids, such as stylet oils (14,15), row covers (23), and reflective mulches (1,29,30,36). Since the

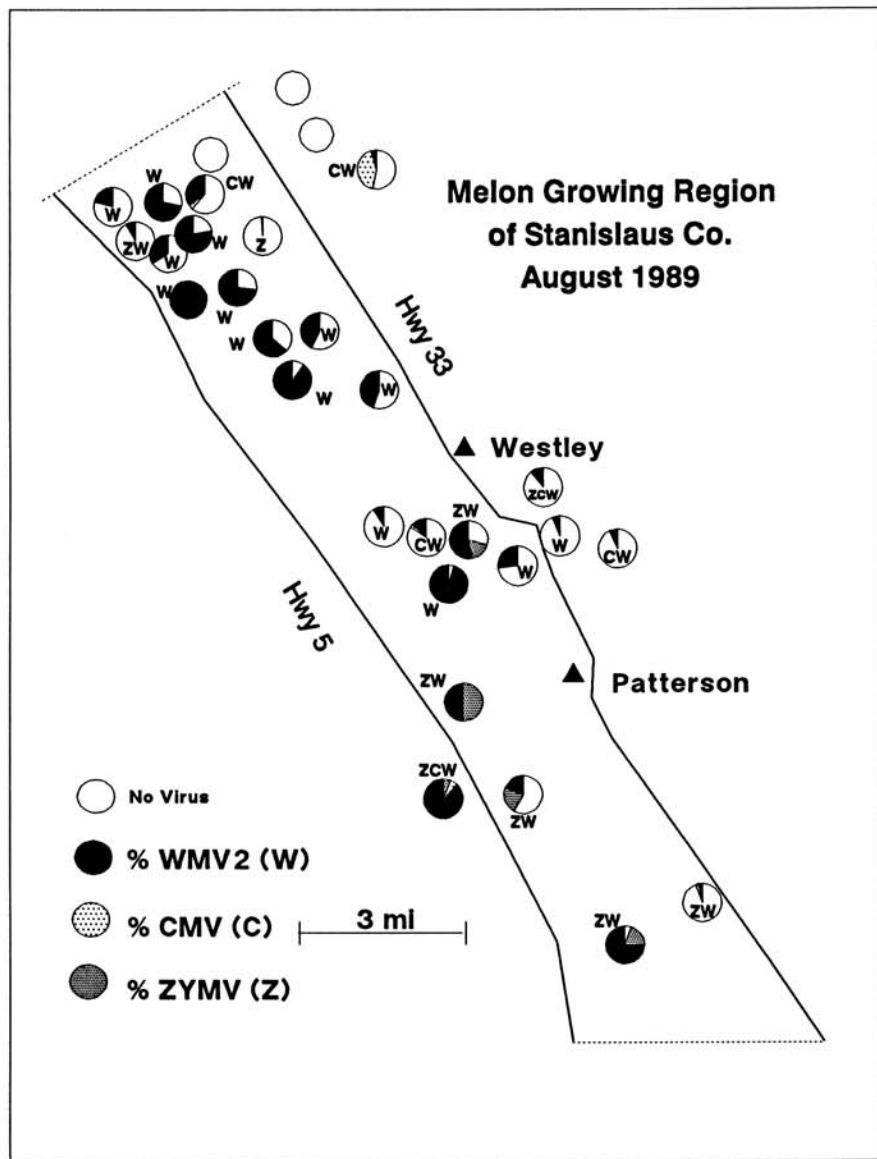


Fig. 4. The distribution of melon fields and the incidence of mosaic symptoms (single infections of watermelon mosaic virus 2 [WMV2], cucumber mosaic virus [CMV], and zucchini yellow mosaic virus [ZYMV]) during 1989 in the Stanislaus County region. The proportions of each virus shown are from the most heavily infected NW or SE corner of each field.

host range of ZYMV is more limited, an areawide effort to eliminate early-season virus sources might prove beneficial to reduce this disease. The situation in the Fresno/Merced counties may be the easiest to manage. In the 2 years of our survey, we found severe disease only late in the 1989 season. Since high infection rates occurred only in the late season, early planting may be a successful strategy for minimizing the impact of epidemics of aphid-borne viruses in this region.

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