Field and Laboratory Transmission of Watermelon Mosaic Virus 2 and Zucchini Yellow Mosaic Virus by Various Aphid Species

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ABSTRACT

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Aphid transmission of watermelon mosaic virus 2 (WMV 2) and zucchini yellow mosaic virus (ZYMV) was studied in the laboratory and field to evaluate species of probable importance in the epidemiology of each virus. Relative transmission efficiencies among species were compared using alates obtained from laboratory-maintained colonies as well as from natural populations collected with an aerial net. In laboratory studies, *Myzus persicae*, *Aphis gossypii*, and *Acyrthosiphon pisum* transmitted WMV 2 with 18, 16, and 16% efficiencies, respectively. In similar studies, these three species transmitted ZYMV with 41, 35, and 4% efficiencies. In these laboratory studies, *Acyrthosiphon kondoi*, *Lipaphis erysimi*, and *Aphis spiraecola* transmitted WMV 2 and ZYMV with less than 10% frequency. Field-collected *M. persicae* alates, whether returned to the laboratory or tested immediately in the field, transmitted ZYMV and WMV 2 at higher efficiencies than the laboratory-derived alates. Field-collected *A. kondoi* and *A. pisum* transmitted ZYMV at higher efficiencies than those evaluated in laboratory studies, but transmitted WMV 2 with lower efficiency. In a related study, a total of 18,837 alates were assayed by transmission during a 3-yr period to determine incidence of naturally occurring inoculum. Viruliferous aphids were detected only in the first season, when 44 alates representing four species transmitted virus. No alates assayed in the following two seasons transmitted virus.

Insight into the spatio-temporal dynamics of virus movement in a crop can be obtained by studying relationships between a virus and its vectors. Field studies that estimate the proportion of inoculative aphids for each vector species can provide a direct measure of the relative contribution of each species to virus movement. However, the proportion of inoculative aphids may be so low in an area that detection is not possible. In such instances, information gathered from laboratory transmission experiments can be useful in discerning probable field vectors of the virus under study.

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The present study was conducted with two aphid-transmitted potyviruses, watermelon mosaic virus 2 (WMV 2), and zucchini yellow mosaic virus (ZYMV). Both viruses cause considerable losses in cucurbit crops in many regions of the world (10), and commonly infect spring plantings of cucurbits in the Imperial Valley, California (11). Previous studies have examined aphid transmission of WMV 2 (6,18) or ZYMV (8) in the laboratory, whereas another study was concerned with detecting aphid inoculativity of either virus from field-collected alates (2). Purcifull et al (13) noted that 38 aphid species have been identified as vectors of WMV 2, whereas seven aphid species have been identified as vectors of ZYMV (2,9). Various techniques have been used in the previous aphid transmission studies, and in many instances the objectives were concerned only with a qualitative identification of those species transmitting WMV 2 or ZYMV.

Although attempts to identify whether a species is capable of transmitting a virus are important, comparisons among the relative transmission efficiencies of different aphid species provide a better opportunity for identifying the important contributors to virus spread. At the same time, investigations into the abundance of naturally occurring inoculative alates within the geographical region of study also may provide an indication of the potential for virus movement.

As part of the process of identifying those aphid species that play a role in the epidemiology of WMV 2 and ZYMV in the Imperial Valley, laboratory and field transmission efficiency experiments were performed in addition to field studies to estimate the proportion of inoculative aphid alates. Information derived from each of these approaches is being considered in the context of relative abundances of different aphid species so that we may evaluate the important vector species of WMV 2 and ZYMV in the Imperial Valley.

MATERIALS AND METHODS

Aphid transmission studies. Laboratory colonies of aphids were established from alates trapped live in Imperial County, CA. A single clonal line of each species was used in all tests. Respective host plants for each species were infested with a single aphid and maintained in separate cages in a greenhouse. *Myzus persicae* (Sulzer) and *Lipaphis erysimi* (Kaltenbach) colonies were reared on *Brassica juncea* (L.) Czernj. & Coss., *Acyrthosiphon pisum* (Harris) and *Acyrthosiphon kondoi* Shinji on *Medicago sativa* L., *Aphis gossypii* Glover on *Cucumis melo* L., and *Aphis spiraecola* Patch on *Apium graveolens* L. Plants of each species were grown from seed in an insect- and virus-free greenhouse and used to replace plants in the aphid colonies on a regular basis, thus assuring vigorous aphids for transmission trials. We relied on natural production of alates in the colonies and used only alates that had left their rearing host.

Putative samples of WMV 2 and ZYMV were collected from infected muskmelon plants in the Imperial Valley and sap-inoculated to a set of differential indicator host plants for each sample. Absence of infection (determined by lack of symptoms and/or aphid transmission) in *Nicotiana glutinosa* L. and *Ranunculus sardous* Crantz and presence (symptoms and/or aphid transmission) in *Pisum sativum* L. 'Alaska Pea' indicated a sample of WMV 2 free of cucumber mosaic virus (CMV) and ZYMV. A ZYMV positive sample was indicated by infection in *R. sardous* and absence of infection in *N. glutinosa* and Alaska Pea. The isolation of WMV 2 and ZYMV from each other and from papaya ringspot virus (PRSV) was confirmed with immunodiffusion tests (12) using antisera specific to each virus.

Isolates of WMV 2 and ZYMV were maintained in squash (*Cucurbita pepo* L.) cv. Early Prolific in a greenhouse restricted for virus cultures. Young squash plants were aphid-inoculated every 4–6 wk to replace virus culture plants that became oversized and to maintain the aphid transmissibilities of the isolates.

Inoculum source plants in all transmission tests were Early Prolific squash with three to five true leaves, aphid-inoculated with WMV 2 or ZYMV 9–12 days before each transmission test. Squash plants in the cotyledon stage were grown individually in 10-cm pots and served as indicator test plants. Each test plant was caged individually during the transmission test to protect against unintentional aphid exposure. Two series of aphid transmission tests were conducted. In series A, *M. persicae*, *A. kondoi*, *A. pisum*, *L. erysimi*, *A. gossypii*, and *A. citricola* were collected from their respective laboratory colonies and used in various combinations in a total of 10 transmission tests (Table 1). The number of treatments in each test varied according to the number of aphid species and number of viruses evaluated. Tests were conducted as adequate numbers of aphids became available. Treatments were randomized within blocks and replicated at least 16 times in each transmission test. A single isolate each of WMV 2 and ZYMV was used in the series A tests. Aphid alates that had departed their colony rearing host were aspirated from the tops of cages, and each species was held separately in 29.6-ml glass jars for a 1- to 2-h preacquisition fasting period. Collection of alates from their cages was staggered so that their preacquisition period would not exceed 2 h. Alates were transferred singly and placed on the inoculum source plant, using a single young leaf for all aphid probings. Each aphid was observed with a ×10 hand lens and allowed a single probe of 10–60 s duration. Only those aphids that terminated probes

TABLE 1. Results of transmission tests (Series A) of isolates of watermelon mosaic virus (WMV) 2 and zucchini yellow mosaic virus (ZYMV) by the alate forms of six species of laboratory-reared aphids[a]

	Transmission test number													Totals	
	1	2	3	4	5	6	7	8		9		10			
Aphids tested	WMV2					ZYMV		WMV 2	ZYMV	WMV 2	ZYMV	WMV 2	ZYMV	WMV 2	ZYMV
Myzus persicae	8/24 0.33	7/24 0.29	...[b]	6/20 0.30	3/28 0.11	9/24 0.38	13/32 0.41	5/20 0.25	15/20 0.75	1/32 0.03	9/32 0.28	1/20 0.05	6/20 0.30	31/168 0.18	52/128 0.41
Aphis gossypii	4/24 0.25	4/24 0.25	9/16 0.56	...	2/28 0.07	10/24 0.42	13/32 0.41	4/20 0.20	...	0/32 0	8/32 0.25	...	...	23/144 0.16	31/88 0.35
Acyrthosiphon kondoi	2/24 0.08	0/24 0	2/16 0.13	0/20 0	0/28 0	0/24 0	0/32 0	...	...	...	...	0/20 0	0/20 0	4/132 0.03	0/76 0
Acyrthosiphon pisum	...	...	3/16 0.19	1/20 0.05	6/28 0.21	0/24 0	2/32 0.06	...	...	...	...	...	...	10/64 0.16	2/56 0.04
Lipaphis erysimi	0/24 0	...	...	...	...	...	...	0/20 0	...	...	...	...	...	0/44 0	...
Aphis citricola	...	2/24 0.08	...	1/20 0.05	...	...	...	...	...	...	...	...	...	3/44 0.07	...

[a]The number of plants infected (numerator) out of the number tested (denominator) are shown for each species and each test. Proportion of plants infected is shown below each fraction.

[b]Not tested.

naturally within the timed interval were transferred to the test plants for their inoculation feeding period. Each aphid was observed feeding before covering test plants individually with cages. Aphids on test plants were left undisturbed overnight, after which the plants were moved to the greenhouse for fumigation with dichlorvos. Cages were removed from plants the following day.

Series B tests used alates of *M. persicae*, *A. kondoi*, and *A. pisum* that were trapped in flight using an aerial net on various days in the Imperial Valley. This aerial net was made of nylon with dimensions 1.2 by 3.9 m and was elevated 1–2 m above the ground and oriented perpendicular to wind direction to intercept aphids in flight. Alates were aspirated from the net and returned to the laboratory where they were maintained on previously uninfested host plants. Because the number of alates of each species varied with each collection date, transmission tests were conducted using only one species per test according to their availability, except for the first test when two species were used. Otherwise, the protocol for series B transmission tests was the same as series A.

One additional transmission test was conducted with field-collected alates. A WMV 2 inoculum source plant and caged test plants were transported to a field house in the Imperial Valley at the site of the aerial net. Aspirated aphids were held in glass jars for at least 15 min before being allowed access to the source plant. Subsequent steps of the transmission test were the same as in series A and B. The following day, each alate was collected from the test plants and identified to species.

Alate assay. Aphid alates were collected beginning at or before muskmelon emergence in 1985 and 1986 at the USDA field station near Brawley, CA (BFS) and the University of California's Imperial Valley Agricultural Center (IVAC) at Meloland, CA. Aphids were collected from the aerial net that was positioned at the upwind edge of a muskmelon field at IVAC, while at BFS net placement was on fallow ground about 100 m from a muskmelon field. Shifting wind direction or calm periods often made the direction of aphid flight from their sources variable and unknown. Sampling of alates at either one or the other locations occurred on a weekly basis. In 1987, alates were collected at both locations on a twice per week schedule beginning in January. In all three years, sampling continued through the muskmelon season until fewer than 25 alates per day could be collected for assaying.

Aphids landing on the net were aspirated throughout the daylight period and transferred individually to caged indicator plants. Squash cv. Early Prolific plants in the cotyledon stage were used as the indicator plants throughout the study. Seeds were germinated in vermiculite in an environmental chamber with a 14 h light/10 h dark cycle and corresponding 35/28 C temperature cycle. When the hypocotyl was 1–3 cm in length, germinated seeds were transplanted into 5-cm peat pots filled with white sand to facilitate aphid recovery. Peat pots were positioned side-by-side into redwood flats in an insect-free greenhouse for transplanting. Seedlings were given 2–3 days to emerge from the pots, then covered individually by ventilated plexiglass cylindrical cages. A 10-mm hole with a removable cork plug in the cap of each cage allowed access to test plants.

Aphids collected from the net in the aspirator jar were picked up one at a time with a camel's hair brush and placed on each indicator test plant. These plants were kept in a darkened room at BFS or a shaded area at IVAC to reduce light stimulus to the aphids and encourage probing or feeding on the plants. On the day following aphid introduction to the plants, cages were removed, one at a time, and the aphid was collected and placed in a well of a microtitre plate filled with 90% ethanol. Each numbered well corresponded to the number of the plant from which the aphid was collected. When all aphids were collected, plants were fumigated with dichlorvos to kill aphid larvae that had been deposited by the alates.

Plants were kept in the greenhouse for 2–3 wk to allow symptom expression of infected plants. If an infected plant was observed, leaves with symptoms were removed, placed in a labeled plastic bag, and frozen for later virus diagnosis. Each frozen sample was tested for ZYMV and WMV 2 using indirect ELISA (enzyme-linked immunosorbent assay) procedures (7) specific for each virus. Each aphid collected from an infected plant was identified to species under a microscope and matched to the virus it transmitted based on ELISA results. All other aphids not transmitting virus also were identified.

RESULTS

Aphid transmission studies. Of the laboratory-reared aphids, *M. persicae* transmitted WMV 2 more frequently than any other aphid species. In a direct comparison with three other species (tests 1,2,4,5) or with two other species (test 8) per test, transmission of WMV 2 by *M. persicae* was highest in four of the five tests (Table 1). However, the difference in transmission of WMV 2 between *M. persicae* and *A. gossypii* was not significant ($x^2 = 0.30$, df = 4, $P = 0.99$) in the five tests (1,2,5,8,9) in which a direct comparison was made. There also was no significant difference between *M. persicae* and *A. pisum* ($x^2 = 0.91$, df = 1, $P > 0.25$) in the two tests (4 and 5) in which a direct comparison was made, nor between *A. gossypii* and *A. pisum* ($x^2 = 2.07$, df = 1, $P > 0.1$; tests 3 and 5) in their direct comparisons. The overall proportions of plants that became infected with WMV 2 due to transmission by *M. persicae*, *A. gossypii*, and *A. pisum* was 0.18 ($n = 168$), 0.16 ($n = 144$) and 0.16 ($n = 64$), respectively. Transmission of WMV 2 by *A. kondoi* or *A. citricola* was not as high as the previously mentioned species, and *L. erysimi* did not transmit WMV 2 (Table 1).

Transmission of ZYMV in the series A tests was higher for *M. persicae* and *A. gossypii* compared to their respective transmission efficiencies of WMV 2 (Table 1). In five tests, the overall transmission of ZYMV by *M. persicae* was 0.41 ($n =$ 128). For *A. gossypii* in three tests, transmission of ZYMV was 0.35 ($n = 88$). In direct comparisons between *M. persicae* and *A. gossypii* (tests 6,7,9), the total transmissions of ZYMV by each species was 31/88 (infections/total number tested), with little or no deviation between the two species in any one test. The

TABLE 2. Results of laboratory transmission tests (Series B) of isolates of watermelon mosaic virus (WMV) 2 and zucchini yellow mosaic virus (ZYMV) by field-collected alates[a]

Species	WMV 2	ZYMV
Myzus persicae	27/77	35/69
	0.35	0.51
Acyrthosiphon kondoi	0/49	5/49
	0	0.10
Acyrthosiphon pisum	0/19	16/91
	0	0.18

[a]The total number of plants infected (numerator) out of the number tested (denominator) is shown for each species. Proportion of plants infected is shown below each fraction.

TABLE 3. Results of transmission tests of watermelon mosaic virus (WMV) 2 by field-collected alates allowed immediate access to a virus source plant[a]

Species	Transmissions	Trials	Proportion
Myzus persicae	26	41	0.63
Aphis gossypii	3	3	1.0
Lipaphis erysimi	0	41	0.0
Acyrthosiphon pisum	0	4	0.0
Acyrthosiphon kondoi	0	3	0.0
Therioaphis maculata	0	7	0.0
Schizaphis graminum	0	1	0.0

[a]The number of plants infected (transmissions) out of the number tested (trials) is shown for each species and each test. Proportion of plants infected also is indicated.

ranges among tests in the proportion of plants infected with ZYMV transmitted by *M. persicae* was 0.28–0.75 and by *A. gossypii* was 0.25–0.42. Laboratory-reared *A. kondoi* failed to transmit ZYMV ($n = 76$) and *A. pisum* transmitted ZYMV at a low rate (0.04, $n = 56$). Transmission of WMV 2 by field-collected alates of *M. persicae* (0.35, $n = 77$) was comparable to the highest level achieved by laboratory-reared *M. persicae* (Table 2). In test one (series B) in which WMV 2 was transmitted by *M. persicae* in eight out of 28 attempts, 19 field-collected *A. pisum* also were tried but without a single transmission of WMV 2 (Table 2). In a separate trial, no *A. kondoi* out of 49 that were tested transmitted WMV 2. All three species, *M. persicae*, *A. pisum*, and *A. kondoi* represented in tests by field-collected alates were more successful transmitting ZYMV than WMV 2 (Table 2). More than half (35/69) of all *M. persicae* transmitted ZYMV. In a single trial, 5/49 *A. kondoi* transmitted ZYMV. The cumulative transmission in three tests of ZYMV by *A. pisum* was 16/91, or 0.18.

Out of seven species that were trapped in flight and then given access immediately to a WMV 2 source plant, only *M. persicae* and *L. erysimi* were represented by substantial numbers of individuals (Table 3). The highest transmission rate of WMV 2 by *M. persicae* obtained in all studies (0.63, $n = 41$) was achieved in the field test. The field-tested *L. erysimi* failed to transmit WMV 2, consistent with the *L. erysimi* laboratory-reared alates. Three *A. gossypii* alates transmitted WMV 2.

Alate assay. Thirty-three species were assayed during the entire study, although many were represented by comparatively few individuals (Table 4). The combined totals of *M. persicae*, *A. kondoi*, *A. pisum*, *L. erysimi*, and *R. padi* accounted for over 91% of all aphids that were assayed. Of these five species, *M. persicae* was predominant on the net and most frequently assayed for virus inoculativity.

A total of 18,837 aphids were assayed for natural virus inoculativity during the study over 3 yr (Table 4). Aphid abundances in the Imperial Valley were lowest in 1986 and correspondingly fewer alates were assayed compared to either 1985 or 1987. Most of the alates assayed in the 3-yr study were collected in 1987 when sampling was initiated earlier in the year, and number of days sampled was more than three and a half times the previous 2 yr combined.

Virus transmission by field-collected alates was observed only in 1985 (Table 4). Of the 2,980 aphids tested for virus inoculativity in 1985, 44 (1.5%) transmitted virus. No inoculative aphids were collected in either 1986 or 1987, even though more than five times as many aphids were assayed in 1987 compared to 1985 (Table 4).

All virus transmissions in 1985 occurred on the last three sampling dates (Table 5). Before 16 April, no infections in the test plants had occurred out of 2,592 alates tested. A preliminary random sampling and subsequent ELISA testing of 60 muskmelon plants from the IVAC field on 5 April detected no infected plants. An assay of 247 alates at IVAC on 10 April also resulted in no infections in the test plants. However, by the time of the next sampling of field plants on 19 April, ELISA analyses of 100 muskmelon plants indicated 98% infected with WMV 2 and 1% infected with ZYMV. Just three days earlier on 16 April at IVAC, 24 aphids out of 281 tested had transmitted WMV 2 during the alate assay (Table 5). Similarly, no transmissions in the alate assay at BFS occurred until a substantial proportion of muskmelon plants in the field were infected. No virus was detected in a random sample of 60 plants at BFS on 6 April, and the next sample of 100 plants on 12 April indicated 0 and 3% incidence for WMV 2 and ZYMV, respectively. An assay of 268 alates conducted at BFS on 12 April resulted in zero transmissions. Inoculative alates were detected the following assay conducted 19 April at BFS with 18 of 64 transmitting WMV 2 to test plants (Table 5). Incidence of WMV 2 and ZYMV in the BFS muskmelon field on 19 April was 28 and 54%, respectively.

TABLE 4. Numbers of each aphid species (field collected) assayed for isolates of watermelon mosaic virus 2 and/or zucchini yellow mosaic virus during the winter-spring periods in 1985, 1986, and 1987

No.	Species	Number of alates assayed			
		1985	1986	1987	Totals
1	*Myzus persicae*	1,711	85	6,410	8,206
2	*Acyrthosiphon kondoi*	433	6	3,320	3,759
3	*Acyrthosiphon pisum*	529	8	1,893	2,430
4	*Lipaphis erysimi*	170	293	1,149	1,612
5	*Rhopalosiphum padi*	60	178	875	1,113
6	*Hyperomyzus lactucae*	10	27	420	457
7	*Schizaphis graminum*	8	18	193	219
8	*Brachycaudus rumexicolens*	3	14	123	140
9	*Brachyunguis bonnevillensis*	0	0	125	125
10	*Macrosiphum rosae*	5	4	112	121
11	*Hyalopterus pruni*	2	60	52	114
12	*Aphis gossypii*	4	4	90	98
13	*Rhopalosiphum maidis*	7	12	77	96
14	*Metopolophium dirhodum*	7	0	62	69
15	*Brevicoryne brassicae*	1	16	36	53
16	*Dysaphis plantanginea*	0	13	18	31
17	*Uroleucon erigeronensis*	2	2	26	30
18	*Aphis craccivora*	5	0	19	24
19	*Brachycaudus helichrysi*	4	3	14	21
20	*Capitophorus elaeagni*	1	6	13	20
21	*Acyrthosiphon lactucae*	0	0	18	18
22	*Pemphigus* spp.	1	2	14	17
23	*Sitobion avenae*	1	0	16	17
24	*Dysaphis tulipae*	2	1	11	14
25	*Therioaphis maculata*	9	0	4	13
26	*Eucarazzia elegans*	4	1	7	12
27	*Aphis armoraceae*	0	0	2	2
28	*Aphis nerii*	0	0	1	1
29	*Rhopalosiphum nymphaceae*	0	0	1	1
30	*Capitophorus hippophaes*	0	0	1	1
31	*Aphis citricola*	0	0	1	1
32	*Brachycaudus cardui*	0	0	1	1
33	*Uroleucon ambrosia*	1	0	0	1
Totals		2,980	735	15,122	18,837
Transmission[a]		44 (.015)	0 (0)	0 (0)	44 (.002)

[a]Number of individuals that transmitted virus out of the total number of aphids assayed. Percentage of transmission is indicated in parentheses.

TABLE 5. Field transmission of watermelon mosaic virus (WMV) 2 and/or zucchini yellow mosaic virus (ZYMV) by four species of aphids in the Imperial Valley, CA, during three dates in 1985[a]

Species	16 April				19 April				3 May			
	Number assayed	Number transmitting		Percentage that transmitted virus[b]	Number assayed	Number transmitting		Percentage that transmitted virus	Number assayed	Number transmitting		Percentage that transmitted virus
		ZYMV	WMV 2			ZYMV	WMV 2			ZYMV	WMV 2	
Myzus persicae	121	0	15	12.4	53	0	13	24.5	1	1	0	100
Acyrthosiphon pisum	135	0	5	3.7	11	0	5	45.4	25	1	0	4.0
Acyrthosiphon kondoi	19	0	3	15.8	0	0	0	0	0	0	0	0
Rhopalosiphum padi	6	0	1	16.7	0	0	0	0	17	0	0	0
Totals	281	0	24	8.5	64	0	18	28.1	43	2	0	4.6

[a]No transmission was observed on any other dates in 1985, 1986, or 1987.
[b]Percentage of alates of each species that transmitted virus.

In contrast to 1985, we observed that virus spread was slower, and the percentage of virus incidence was less for equivalent dates in 1986 at BFS and 1987 at both locations (4). At IVAC in 1986, incidences of both viruses were early and high, but aphid aerial densities were too limited for sufficient sampling. Aphid abundances in 1986 and 1987 compared to the same dates in 1985 were much lower, especially for those species that had been confirmed as field vectors in 1985. *M. persicae*, *A. pisum*, *A. kondoi*, and *Rhopalosiphum padi* were demonstrated to be field vectors of WMV 2, whereas only *M. persicae* and *A. pisum* transmitted ZYMV in the alate assay (Table 5).

DISCUSSION

Combining field and laboratory experiments permitted a more robust determination of probable vectors of WMV 2 and/or ZYMV than either approach by itself. The field assay of alates extended results of laboratory transmission experiments by showing *M. persicae*, *A. pisum*, and *A. kondoi* to be field vectors of one or both viruses. These data are consistent with similarly conducted studies (2) that found higher transmission of ZYMV than WMV 2 by *M. persicae*, and transmission of ZYMV coupled with no transmission of WMV 2 by *A. pisum*. Adlerz (2) did not evaluate *A. kondoi*. Moreover, *R. padi* was demonstrated in the present study to be a field vector, whereas transmission in the laboratory trials did not occur because *R. padi* would not probe infected squash source plants. Field assays detected aphids that were inoculative during one of three years, and then only on the last three sampling dates after virus incidence was high in muskmelon plants of surrounding fields. Because of the rarity of inoculative alates assayed in the field, data obtained in the laboratory on the transmission of WMV 2 and ZYMV provided useful information on probable vectors of these two viruses in the Imperial Valley.

Although variation was observed in transmission results among tests, consistency among the ranking of species throughout all tests was maintained. For example, in the five tests (1,2,5,8,9; Table 1) in which *M. persicae* and *A. gossypii* were compared directly in the transmission of WMV 2, *M. persicae* consistently transmitted at a slightly higher rate than *A. gossypii* (difference of 0.03–0.08), even though the proportion of test plants infected by each species varied considerably among tests (0.03–0.33 for *M. persicae*, 0–0.25 for *A. gossypii*). Although field-originated *A. gossypii* were not available for testing in our studies, Adlerz (2) found that field-collected *M. persicae* transmitted WMV 2 with 20.0% efficiency, whereas *A. gossypii* did not transmit either virus in his studies. Laboratory studies that attempt to determine relative vectoring efficiencies of different aphid species often use single clonal lines of each species to minimize potential variation due to putative genetic differences. However, reports of variation in ability to transmit a virus among different clones of an aphid species are common (3,14–17). It is possible that the ability of a species to transmit a given virus may be misrepresented if only a single clone is tested. Therefore, we conducted transmission experiments with aphids collected in the field to supplement data collected in the laboratory. In this study, alates of *M. persicae* transmitted both WMV 2 and ZYMV at a higher overall level than laboratory-reared aphids. Previous studies (2) have found transmission of WMV 2 and ZYMV by *M. persicae* to be less than aphids tested in our studies. Field-originated *A. pisum* and *A. kondoi* transmitted ZYMV at higher rates than did laboratory-reared aphids, but laboratory aphids transmitted WMV 2 at higher efficiencies.

Results of transmission studies that utilize single isolates of the test virus and single clones of the test aphid species are meaningful within the framework of the immediate experiment only. Generalizations based on experience with a single clone and single virus isolate concerning relative transmission efficiencies of aphids are tenuous. Broader understanding of the vectoring ability of an aphid species is realized when a consistent pattern of transmission efficiency results using aphids and virus isolates from different sources. The relative ranking of *M. persicae*, *A. pisum*, and *A. kondoi* in the two series of tests in this study was consistent even though intraspecific variation between tests was high. Moreover, the close similarities between *M. persicae* and *A. gossypii* in their respective vectoring abilities of WMV 2 agrees with previous reports (1,6). The consensus of information from the present and former studies support the generalization of *M. persicae* and *A. gossypii* as efficient vectors of WMV 2 and ZYMV.

Assays for virus inoculativity of field-collected alates encompass many of the factors, operating at a point in time, that affect the probability of a single aphid vectoring virus. With the exceptions of interception of the aphid in flight and confinement to a young test plant, little compromise of natural conditions is involved in understanding which aphids are involved in virus spread and what proportion of these aphids are viruliferous.

Although the basis for assaying alates in an epidemiological investigation is intuitively sound, the effort required to assay sufficient numbers of alates to detect a few or more transmissions is, as Irwin and Ruesink (5) described, "staggering." From the example of the 1985 season in this study, it was apparent that a high incidence of infected plants and the presence of a sufficient number of alates for sampling was necessary to detect inoculative aphids. In 1986 and 1987, inoculative aphids were not detected because aphid numbers had declined to an insufficient level for assaying by the time virus incidence had increased to a high level (Table 4, 1986), or virus incidence remained limited throughout the season irrespective of the availability of aphids (Table 4, 1987).

The low abundance of inoculative aphids provided some perspective on the epidemiology of WMV 2 and ZYMV in muskmelon fields in southern California. The rarity of inoculative aphids, especially before the time of widespread incidence of infected muskmelon fields, suggests that primary virus transfer from external sources into melon fields occurs with low frequency. Once an initial infection is established in a field, secondary spread within the field and to adjacent fields can occur rapidly providing there is substantial vector activity. Previous studies indicate that high aphid densities occur during most spring melon growing seasons in the Imperial Valley (4), which would account for the high incidence of these viruses typical in the area.

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