Alteration of Efficiencies of Acquisition and Inoculation of Watermelon Mosaic Virus 2 by Plant Resistance to the Virus and to an Aphid Vector

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ABSTRACT

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The effects of aphid and virus resistance on acquisition and inoculation of watermelon mosaic virus 2 (WMV 2) were assessed using a colonizing (*Aphis gossypii*) and a noncolonizing (*Myzus persicae*) aphid species and three *Cucumis melo* genotypes. Together, these provided a treatment set containing all four combinations of plant resistance or susceptibility to aphids with resistance or susceptibility to virus. Plant resistance to WMV 2 reduced the efficiency of virus acquisition by both aphid species but had no detectable effect on inoculation efficiency. Virus acquisition efficiency was a function of virus concentration in the source plant and affected the probability of virus transmission by both aphid species similarly. Resistance to aphids reduced the efficiency of inoculation by *A. gossypii*, the resisted aphid, but not by *M. persicae*. Resistance to inoculation by *A. gossypii* without resistance to acquisition in the same genotype suggests that the requirements for aphid inoculation of viruses may differ from those for acquisition.

Watermelon mosaic virus 2 (WMV 2) occurs worldwide and causes significant losses in *Cucurbita pepo* L. and *Cucumis melo* L. Resistance to the virus has not been reported in these agronomically important species until recently, when a suppressive form of resistance that restricts multiplication of WMV 2 was identified in the *C. melo* accession 91213 and was shown to reduce WMV 2 spread under field conditions (13). This accession also possesses high levels of resistance to *Aphis gossypii* (Glover). This resistance, specific to this aphid, is both antibiotic (i.e., aphid survival and reproduction are reduced) and antixenotic (i.e., aphids do not settle and feed readily) (6,7). Although definitive genetic studies have not been performed, we suspect that resistance to inoculation of viruses is a pleotropic effect of genes conditioning resistance to *A. gossypii*, as in Lecoq et al (10) and Pitrat and Lecoq (14).

Although virus resistance that reduces virus concentration has long been recognized as a potential means of limiting virus spread (1), there are few documented examples of the use of partial resistance to viruses or to their vectors in controlling the spread of plant viruses, particularly those that are nonpersistently transmitted (1,4,9,12,13,18). Little is known of how different types of resistance affect the individual components (acquisition, retention, and inoculation) of the plant-virus-vector interactions that determine virus spread (23). Examples suggest that different types of virus resistance may either inhibit virus multiplication and thereby reduce the amount of virus available for acquisition or inhibit infection; both types reduce transmission efficiency (17,18,21).

Resistance to aphid vectors alone has generally been considered of limited effectiveness in reducing the spread of nonpersistently transmitted viruses unless the resistance prevents aphid probing of the potential host or greatly reduces vector populations (4,5). However, there are examples of aphid resistance associated with reduced virus-transmission efficiency and thus reduced spread (3,9,11,21).

The aphid-virus-plant system we have investigated is similar to that studied by Lecoq et al (9,10) and Risser et al (16) in which they found that genes conferring resistance to *A. gossypii* in *C. melo* also selectively inhibited inoculation by *A. gossypii* of CMV, WMV 2, WMV 1, and muskmelon yellow stunt virus. Our research extends their findings and provides a more detailed description of the combined effects of suppressive virus resistance and resistance to inoculation by *A. gossypii* on transmission of WMV 2. We estimated the effects of aphid and virus resistance, occurring separately and together, on the processes of acquisition and inoculation of WMV 2 by using a colonizing (affected by resistance factors) and a noncolonizing (not affected by resistance factors) aphid species on aphid- and virus-resistant and susceptible *C. melo* genotypes.

MATERIALS AND METHODS

Experiments were designed to examine two components of the transmission process, acquisition and inoculation, separately and together. In all experiments, we estimated the overall rate of transmission, which incorporates the efficiencies of acquisition, retention, and inoculation. By holding, in turn, two parts of the transmission process constant, we estimated differences in rates of transmission that reflected differences only in the third component. Immediately after an acquisition probe, all aphids were transferred to the plant to be inoculated, thus holding retention time constant. To compare acquisition efficiencies, we used squash as a common recipient. To compare inoculation efficiencies, we used the same virus-source leaf for all recipient melon genotypes in each replicate. To compare intragenotypic virus transmission (as is the case in secondary spread), the virus source leaf was of the same genotype as the recipient plant.

Plant material. Three muskmelon genotypes were used. Top Mark is a commercial cultivar susceptible to both WMV 2 and *A. gossypii*. Accession 91213 is an eighth-generation inbred derived from accession PI 371795 (the same parent as that of the aphid-resistant genotype of Lecoq et al). Accession 91213 has both

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antibiotic and antixenotic resistance to *A. gossypii* (6,7) and suppressive resistance to WMV 2 (13). Aphid-Resistant Top Mark (AR-Top Mark, developed by A. N. Kishaba and G. W. Bohn at USDA Boyden Entomological Laboratories, Riverside, CA) was developed by selecting for resistance to *A. gossypii* in a backcross-breeding program using Top Mark as the recurrent parent and 91213 as the source of aphid resistance. AR-Top Mark and Top Mark are equally susceptible to WMV 2 (13). The aphid resistance in AR-Top Mark and 91213 is specific to *A. gossypii*, the colonizing aphid, and has not been found to have any effect on other sucking insects (2). Because *Myzus persicae* Sulzer, another vector of WMV 2 in muskmelon, does not colonize muskmelon, it was assumed to be "blind" to these aphid-resistance factors.

Because AR-Top Mark is similar to Top Mark except for the gene(s) conditioning aphid resistance, a comparison of transmission of WMV 2 by *A. gossypii* from or to these two cultivars permitted estimation of the effects of aphid resistance alone on acquisition or inoculation, respectively. Melon genotype 91213, having resistance to both WMV 2 and to *A. gossypii*, was compared with AR-Top Mark to determine the effects of virus resistance on acquisition and inoculation of WMV 2 by *A. gossypii*. Because *M. persicae* is unaffected by the aphid-resistance factor, the effects of virus resistance alone can be determined for this vector by comparing acquisition or inoculation by this vector from or to virus-susceptible Top Mark and AR-Top Mark with that from or to virus-resistant 91213. Table 1 summarizes the aphid and virus resistance properties of the different melon genotypes.

Aphid and virus cultures. Single-clone colonies of *A. gossypii* and *M. persicae* were reared on *C. melo* 'Hale's Best' (muskmelon) and *Brassica juncea* L. 'Florida Giant' (mustard), respectively. Both aphid colonies were maintained at 26 C with a 16-hr-light/8-hr-dark cycle.

A single WMV 2 isolate from naturally infected *C. pepo* 'Yellow Crookneck' (squash) (13,15) was maintained in *Pisum sativum* L. and, through aphid inoculation, in Hale's Best muskmelon or yellow crookneck squash held in Saran-cloth cages. In tests comparing efficiency of acquisition from susceptible and virus- and/or aphid-resistant sources, source plants were mechanically inoculated from an aphid-inoculated source 10–24 days before use. For estimating the effects of resistance in the recipient on inoculation efficiency, the virus source was either Hale's Best muskmelon or squash 2–5 wk after aphid inoculation.

Samples of leaves used as virus sources were diluted 1:100 in buffer solution and assayed by enzyme-linked immunosorbent assay (ELISA) to obtain an index of virus titer (13). For all ELISAs, all preparations of coating and conjugate immunoglobins were standardized against each other. For each experiment, only one lot number of plates was used; for each replicate of each experiment, one plate was used. The relationship of titer to absorption values (A_{405nm}) was linear for absorption values between 0.1 and 1.0 (Fig. 1), in which range all but four virus-source leaves fell (three were lower and one was slightly higher). (Aggregation of WMV 2 particles in purified samples precluded using a standard concentration of virus in each serological analysis.) The mean absorbance (A_{405nm}) from four wells was used in the statistical analyses.

Aphid transmission of virus. The virus source for each replicate of each experiment was half of a mature, virus-infected leaf of the appropriate *C. melo* genotype or squash; the opposite half was assayed by ELISA. The third to fifth leaf from the apex was used in all tests. Leaves were kept fresh on moist paper towels in petri dishes at 4 C until use.

Apterous adults of both species were used in all experiments. They were starved in petri dishes for 0.3–1 hr at 26 C, then held (< 3 hr) over ice until they were used in transmission experiments in a multiple-aphid transfer design (3). Each aphid was allowed an acquisition probe of 5–10 sec on a virus-source leaf, then transferred to the first true leaf of a 10- to 14-day-old test plant. The number of aphids per plant was the same for each plant in each treatment replicate.

After 2–12 hr, aphids on all test plants in all experiments were killed by spraying with nicotine sulfate. In all experiments, the number of symptomatic plants was determined 10–14 days after inoculation. In early tests, visual classification of plants as infected or healthy was verified serologically. Only plants with visible symptoms gave positive results in immunodiffusion tests, so we later relied on visual determination alone.

Virus acquisition. To compare acquisition efficiencies, the probability of transmission from Top Mark, AR-Top Mark, and 91213 to squash was estimated by transferring five aphids to each squash plant after each aphid had made an acquisition-access probe on one WMV 2-infected melon genotype. In each replicate, 40 (in three cases, 39) recipient squash plants were inoculated per treatment (i.e., acquisition source × aphid species). Because of time constraints, not all treatments in some replicates could be completed in 1 day, but each replicate, which included all treatments, was completed within 4 days. There were 10 replicates using a total of 2,000 *A. gossypii* per melon genotype used as a virus source; *M. persicae* was included in six of those replicates (1,200 *M. persicae* per source genotype). The same source leaves were used for both aphid species on the same day in comparative studies.

Virus inoculation. To determine the effects of aphid and virus resistance on inoculation efficiency, the probability of transmission by a single aphid was estimated using a common virus source and the three *C. melo* genotypes as recipients. To compare recipient treatments, each replicate was completed in 1 day. In the first 10 replicates of this experiment, *A. gossypii* was the only vector and both yellow crookneck squash and Hale's Best melon

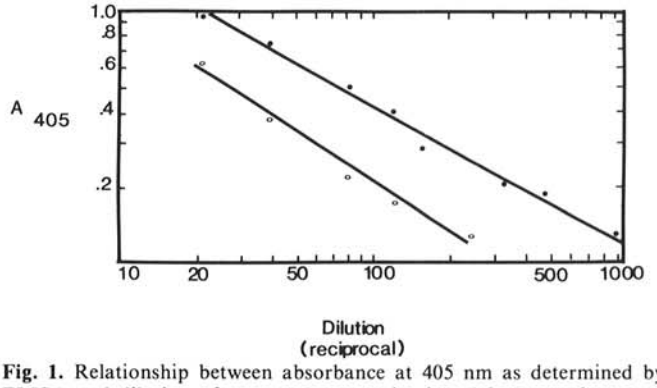

Fig. 1. Relationship between absorbance at 405 nm as determined by ELISA and dilution of watermelon mosaic virus 2 in leaves from two *Cucumis melo* genotypes. • = Top Mark and o = 91213.

TABLE 1. Factorial set of treatments obtained by combining three *Cucumis melo* genotypes possessing different combinations of resistance to watermelon mosaic virus 2 and *Aphis gossypii* with two aphid species, *A. gossypii* and *Myzus persicae*, and used to examine acquisition and inoculation efficiencies

Muskmelon genotype and its resistance properties	Aphid species	
	A. gossypii	*M. persicae*
Top Mark No resistance properties	Susceptible control	Susceptible control
Aphid-Resistant Top Mark Antibiosis and antixenosis to *A. gossypii*	Aphid resistance	Susceptible control
91213 Antibiosis and antixenosis to *A. gossypii* and suppressive resistance to WMV 2	Aphid resistance and virus resistance	Virus resistance

were used as virus sources in each replicate. Squash had low virus titers ($\bar{x}_{A_{405nm}} = 0.161$, $n = 9$, SE = 0.038), whereas Hale's Best had relatively high titers ($\bar{x}_{A_{405}} = 0.516$, $n = 9$, SE = 0.080). (Virus titer was not measured in one replicate, hence $n = 9$.) Between 21 and 40 plants of each melon genotype were inoculated with virus from each source in each replicate. Five aphids were placed on each test plant (except for three per Top Mark plant in seven replicates with Hale's Best virus source).

To compare inoculation of the three melon genotypes by *M. persicae*, nine additional replicates were run using only Hale's Best melon as a virus source. Four of these replicates also included *A. gossypii* with both aphid species using the same Hale's Best virus-source leaf. When only *M. persicae* was used as a vector, 40 plants of each genotype (in one case, 39) were inoculated in each replicate. When both aphid species were used, between 20 and 33 plants of each genotype were inoculated by each aphid species in each treatment replicate. Five aphids were put onto each test plant. In total, more than 4,000 *A. gossypii* and 1,360 *M. persicae* were transferred to plants of each genotype.

Intragenotypic transmission. The effects of aphid and virus resistance on WMV 2 transmission between plants of each genotype was measured by using the same *C. melo* genotype as source and recipient for both aphid species. Each of the six replicates was completed within 4 days. In the first two replicates, five aphids were put onto each test plant. In subsequent replicates, the number of aphids put onto a Top Mark plant was reduced to three while the number of *A. gossypii* put onto 91213 and AR-Top Mark was increased first to 10, then 15, then 20; the number of *M. persicae* put onto 91213 was similarly increased. The total number of aphids transferred to each *C. melo* genotype ranged from 815 *M. persicae* for Top Mark to 2,700 *A. gossypii* for AR-Top Mark.

Statistical analysis. Comparisons of rates of transmission were based on the estimated probability of transmission by an individual aphid for each replicate of each treatment in this multiple-aphid transfer design (20). The probability (P_T) of an individual aphid transmitting WMV 2 was estimated using the maximum likelihood estimator (3,20) as

$$P_T = 1 - (1 - S/N)^{1/K},$$

where N = number of plants tested, S = number of plants that became infected, and K = number of aphids per plant.

Differences among melon genotypes unadjusted for differences in virus titer were determined from analyses of variance using P_T as the dependent variable. The specific questions asked, whether aphid and/or virus resistance affected acquisition, inoculation, or intragenotypic efficiency of WMV 2, were addressed in a series of contrasts (19) (Table 2).

The relationship of the dependent variable, probability of transmission, to titer of the virus source (as measured by ELISA, A_{405nm}) was determined for each treatment (melon genotype × aphid species) within each experiment. Linear and quadratic models were fitted to both untransformed and ln-transformed data [$\ln(P_T + 0.001)$] to determine the best-fitting statistical models.

Comparison of treatments was also based on differences among slopes of the regression lines obtained when titer (A_{405nm}) was included as a covariate in the analyses of variance. That is, the interaction of treatments and the covariate was used to test homogeneity of the slopes. The variable "aphid species" was included in the models to determine whether the response was vector-dependent.

RESULTS

Acquisition. Whether acquisition efficiency of *A. gossypii* was affected by virus resistance in the virus source was addressed by comparing the probabilities of transmission (P_T) to squash when WMV 2 was acquired from 91213 versus from AR-Top Mark (Table 2). (This comparison avoids the confounding factor of aphid resistance.) For *M. persicae*, this question was addressed by comparing the probability of transmission from 91213 versus that from Top Mark and AR-Top Mark. For both *A. gossypii* and *M. persicae*, virus resistance reduced the probability of transmission (Table 2).

Whether aquisition efficiency was affected by aphid resistance was tested by comparing the probabilities of transmission by *A. gossypii* from Top Mark and from AR-Top Mark. There was no difference (Table 2). The comparison of P_T by *M. persicae* from these two sources also showed no difference between these genotypes. Overall probabilities of transmission from the different genotypes by *A. gossypii* and *M. persicae* (averaged across melon genotypes) were not different, nor was there an interaction between aphid species and melon genotype in this 2 × 3 factorial treatment set, indicating that virus resistance in the source affected the acquisition efficiency of both species similarly.

Virus-resistant 91213 plants had lower virus titers, as indicated by lower ELISA readings, than did the other two melon genotypes (see the distribution of points in Figs. 2 and 3). Whether reduced

TABLE 2. The effects of aphid and virus resistance in *Cucumis melo* on acquisition, inoculation, and intragenotypic transmission of watermelon mosaic virus 2

			Probabilities of transmission[a] for comparing			
				Inoculation		
Property tested	Aphid species	Plant genotypes contrasted	Acquisition[b]	Hale's Best melon[c]	Squash[c]	Intragenotypic transmission
Virus resistance	*Aphis gossypii*	Aphid-Resistant Top Mark	0.105 (10, 0.020)*[d]	0.012 (14, 0.004)	0.002 (10, 0.002)	0.014 (0.007)*
		vs.				
		91213	0.036 (10, 0.012)*	0.007 (14, 0.004)	0.002 (10, 0.001)	0.000 (–)*
	Myzus persicae	Top Mark and AR-Top Mark	0.109 (12, 0.020)*	0.076 (18, 0.015)		0.168 (0.028)*
		vs.				
		91213	0.020 (6, 0.007)*	0.084 (9, 0.029)		0.034 (0.009)*
Aphid resistance	*A. gossypii*	Top Mark	0.108 (10, 0.029)	0.109 (14, 0.025)*	0.043 (10, 0.011)*	0.146 (0.017)*
		vs.				
		AR-Top Mark	0.105 (10, 0.020)	0.012 (14, 0.004)*	0.002 (10, 0.002)*	0.014 (0.007)*
	M. persicae	Top Mark	0.091 (6, 0.031)	0.080 (9, 0.022)		0.183 (0.034)
		vs.				
		AR-Top Mark	0.126 (6, 0.025)	0.071 (9, 0.021)		0.152 (0,045)

[a] Means, with numbers of replicates and standard errors in parentheses, of probability of transmission by a single aphid; for each replicate estimated by $1 - (1 - S/N)^{1/K}$, where S = number of virus-infected plants, N = number of plants tested, and K = number of aphids per plant.
[b] Transmission to squash.
[c] Virus source.
[d] Asterisk indicates that paired treatments are different at 5% significance level by LSD or Student's t test.

availability of virus, as measured by lower titer (A_{405nm}), could explain the lower probability of transmission from virus-resistant 91213 was tested by covariance analysis with the different melon genotypes as the class variable and virus source titer as the covariate. In this analysis, the adjusted mean probabilities of transmission from the different genotypes were not different for either aphid species. Furthermore, the slopes of the regression lines relating P_T to titer were similar for the three *C. melo* genotypes within each aphid species, that is, there was no interaction between melon genotype and titer for either aphid species.

To further explore the nature of the relationship between titer (A_{405nm}) and probability of transmission (Figs. 2 and 3), the data for all melon genotypes were pooled within each aphid species. To determine the best statistical model for these pooled data, the untransformed (P_T) and then the transformed [$\ln(P_T + 0.001)$] data were fitted with linear and quadratic models. Statistical comparison of linear and quadratic models showed that the quadratic component did not improve fit for either *A. gossypii* or *M. persicae*. For both aphid species, the linear models using the transformed data fit slightly better than those using the untransformed data (based on a comparison of R^2s); however, because the biological implications of such a model have not been investigated, we report the results of analyses of variance using the untransformed data. In all instances, the same conclusions in comparisons of treatments were reached whether the analyses used the transformed or untransformed data.

Inoculation. Contrasts like those used when examining the effects of aphid and virus resistance on acquisition efficiency were used to determine whether aphid and/or virus resistance affected virus inoculation efficiency. These contrasts showed that virus resistance in the recipient had no effect on inoculation efficiency for either aphid species (Table 2). However, resistance to *A. gossypii* reduced the frequency of inoculation ($P < 0.001$) by *A. gossypii* (Table 2). This difference was considerable, 10- to 20-fold. The same comparison, but using *M. persicae* as a vector to verify that resistance was specific to *A. gossypii*, showed no effect on the probability of transmission by the unresisted vector (Table 2).

The positive correlation between virus titer in the source and probability of transmission was reconfirmed by the regression of probability of transmission on titer for *M. persicae* (slope = 0.20); this was similar to that seen in the acquisition experiments. Additionally, there was a greater ($P < 0.001$) probability of transmission by *A. gossypii* to all melon genotypes from the higher virus titer Hale's Best melon source than from the lower titer squash source. The WMV 2 titer (A_{405nm}) in squash ranged from 0.017 to 0.358; for Hale's Best melon, it ranged from 0.203 to 1.168. When transmission from Hale's Best leaves with titers less than 0.40 (three replicates) was compared with transmission from squash, there was no difference between sources in probability of transmission.

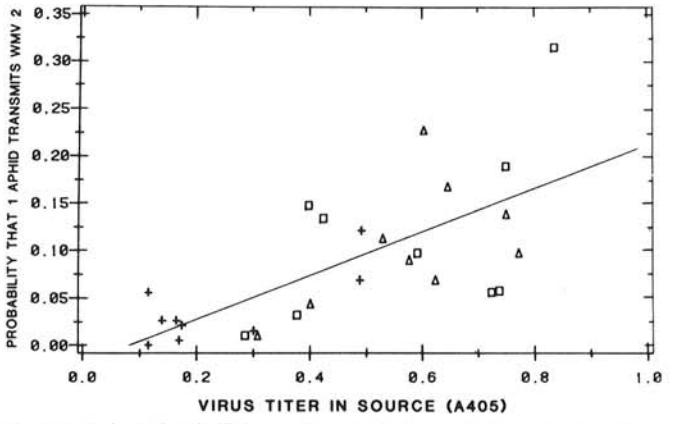

Fig. 2. Relationship of efficiency of transmission by *Aphis gossypii* to virus titer in the source when acquiring watermelon mosaic virus 2 from three *Cucumis melo* genotypes. □ = Aphid- and virus-susceptible Top Mark, Δ = aphid-resistant Top Mark, and + = aphid- and virus-resistant 91213 (slope = 0.23, SE = 0.05; intercept = −0.02, SE = 0.03).

For *A. gossypii*, there was an interaction ($P < 0.001$) between virus titer in the source and the genotype of the recipient for the variable P_T. The nature of this interaction is illustrated in Figure 4, where the slopes of the regression lines relating probability of transmission (P_T) to titer (A_{405nm}) for inoculating the aphid-resistant genotypes (AR-Top Mark and 91213) were lower than for Top Mark, the aphid-susceptible genotype. When the probability of transmission by *A. gossypii* was analyzed using $\ln(P_T + 0.001)$, which reflects proportional rather than absolute changes in probability of transmission for each genotype, the relative difference between the slopes of the regression lines for the different melon genotypes was greatly reduced and the interaction was no longer significant. This suggests that the proportional increase in the probability of transmission with increased titer in the virus source does not differ among the three melon genotypes.

Intragenotypic transmission. The probability of transmission between plants of the same melon genotype (Table 2) was consistent with what would be expected on the basis of the previous estimates of probabilities of transmission to and from each genotype. The probability of transmission by *A. gossypii* between AR-Top Mark plants was only 1/10th of that between Top Mark plants, the same reduction in transmission efficiency as seen in the inoculation experiment. The acquisition and inoculation experiments predicted near-zero probability of transmission between 91213 plants. Although transmission of WMV 2 between 91213 plants was attempted with 2,170 *A. gossypii* adults, none of the plants became infected.

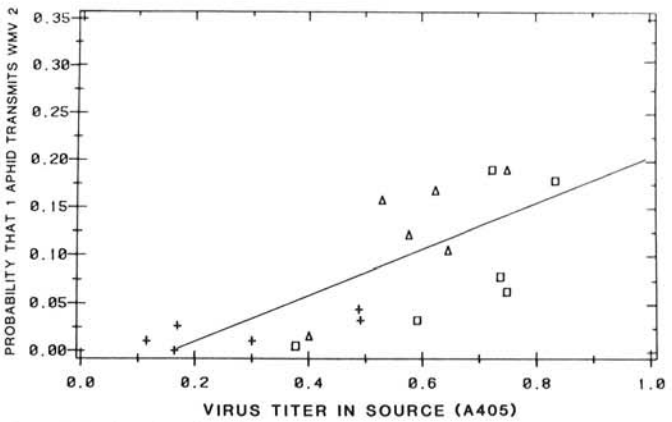

Fig. 3. Relationship of efficiency of transmission by *Myzus persicae* to virus titer in the source when acquiring watermelon mosaic virus 2 from three *Cucumis melo* genotypes. □ = Aphid- and virus-susceptible Top Mark, Δ = aphid-resistant Top Mark, and + = aphid- and virus-resistant 91213 (slope = 0.24, SE = 0.05; intercept = −0.04, SE = 0.03).

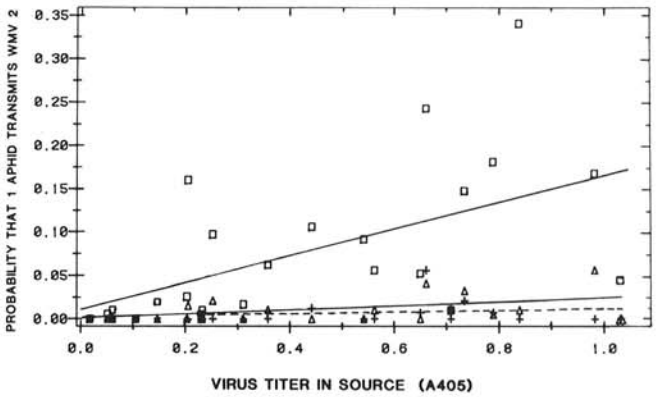

Fig. 4. Relationship of efficiency of transmission by *Aphis gossypii* to virus titer in the source when inoculating three *Cucumis melo* genotypes with watermelon mosaic virus 2. □——□ = aphid- and virus-susceptible Top Mark (slope = 0.15, SE = 0.05; intercept = 0.01, SE = 0.03), Δ——Δ = aphid-resistant Top Mark (slope = 0.02, SE = 0.01; intercept = 0.00, SE = 0.005), and +- - -+ = aphid- and virus-resistant 91213 (slope = 0.001, SE = 0.008; intercept = 0.00, SE = 0.004).

The probability of transmission by *M. persicae* between 91213 plants was about one-fifth that between Top Mark or AR-Top Mark plants. This difference can be attributed, at least in part, to differences in WMV 2 titer in the source plants (mean titer [A_{405nm}] for Top Mark = 0.624 [SE = 0.041], AR-Top Mark = 0.601 [SE = 0.051], 91213 = 0.233 [SE = 0.035]). Adjustment for differences in virus titer by analysis of covariance eliminated differences in efficiency of transmission by *M. persicae* among melon genotypes.

DISCUSSION

Our findings demonstrate that resistance to WMV 2 in 91213 reduces the efficiency of transmission by reducing the acquisition of this nonpersistently transmitted virus by both aphid species studied. The nonspecific nature of the relationship between virus titer in the source and probability of transmission from that source was confirmed by the correlation between virus titer and efficiency of acquisition from both the virus-susceptible and resistant genotypes and the similar slopes of the regression lines for both aphid species (Figs. 2 and 3). Our results do not allow us to determine whether fewer aphids acquired a sufficient amount of virus from the virus-resistant 91213 source leaves to make infection likely or whether aphids generally acquired less virus, thereby lowering the probability of transmission for each individual.

The resistance to WMV 2 in 91213 is different in its effect on the pattern of multiplication and symptom development (13) from that described for some other potyviruses where resistance delays or reduces the virus multiplication rate and delays symptom expression (e.g., 18,22). Virus multiplication in 91213 initially occurs at a rate similar to that in the virus-susceptible genotypes, but the maximum virus titer reached is lower. However, the influence of virus resistance in 91213 on acquisition efficiency is similar to that reported for these other forms of virus resistance that reduce virus titer (9,22).

Resistance to WMV 2 has also been observed to reduce the spread of WMV 2 under some field conditions (13; *unpublished*). Because this form of resistance is not specific to particular vector species, it may be particularly effective in reducing virus spread in the field, although, in small field plots late in the summer, high vector pressure has been seen to offset the benefits of reduced virus-acquisition efficiency attributable to this partial virus resistance (Gray, *unpublished*). An increasing number of examples suggest that partial virus resistance is effective in reducing virus spread under certain field conditions (1,11,13,22).

The resistance to inoculation in aphid-resistant *C. melo* genotypes found here and elsewhere (9,10,16) is specific to *A. gossypii*. This resistance is known to alter the probing behavior of *A. gossypii* (8) and may reduce the rate of inoculation of nonpersistently transmitted viruses by interfering with the ability of *A. gossypii* to deposit virus in sites appropriate for initiation of virus infection. The number of infective sites apparently does not differ between aphid-resistant and aphid-susceptible plants, because all three genotypes were inoculated at similar rates by *M. persicae* (Table 2). That the aphid resistance of AR-Top Mark does not interfere with virus acquisition by either aphid species suggests that the requirements for virus inoculation by aphids are more stringent (or at least different) than are those for virus acquisition.

The information on resistance to *A. gossypii* in 91213 (2,6–8), together with the experimenter's ability to alter the proportion of aphid-resistant (AR-Top Mark or 91213) plants that become infected by changing the number of aphids per plant, indicates that aphid resistance reduces the probability of transmission by reducing the probability for each individual aphid of placing a sufficient quantity of virus in infection sites. The ability to obtain more frequent infection of aphid-resistant plants by increasing the number of viruliferous aphids probing each resistant plant occurs because the likelihood of observing a rare event (infection) is increased by increasing the number of attempted transmissions. It is therefore misleading to state that resistance to aphid transmission is "broken" by mass transfers of individual aphids to resistant plants, as has been suggested (9).

Although resident *A. gossypii* populations on 91213 and AR-Top Mark are much smaller than those on aphid-susceptible genotypes such as Top Mark, there is more interplant movement of *A. gossypii* on the aphid-resistant lines (6,7). *A. gossypii* resistance in another *C. melo* cultivar, Songwhan Charmi, has also been associated with increased aphid activity and resistance to inoculation of nonpersistently transmitted viruses by *A. gossypii* (9,10,16). The *Ag* gene (for *A. gossypii*) is known to condition resistance in 91213 and is similar in effect to the gene responsible for resistance to *A. gossypii* in Songwhan Charmi (9,10,14). Although increased interplant movement of aphids attributable to aphid resistance might be expected to result in increased virus spread (5), our observations (*unpublished*) of spread of WMV 2 in field plantings of Top Mark, AR-Top Mark, and 91213 in North Carolina indicate that the combined effects of suppressive virus resistance, antibiotic aphid resistance, and resistance to inoculation of WMV 2 can offset the effects of increased interplant movement of *A. gossypii* and can provide excellent potential for controlling WMV 2 spread under low vector pressure or when *A. gossypii* is the primary vector.

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