Tobacco Mosaic Virus: Can Aphids Inoculate It Into Plants With Their Mouthparts?

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ABSTRACT

The aphids *Myzus persicae* and *Macrosiphum euphorbiae* failed to inoculate plants with tobacco mosaic virus in several experiments in which they were allowed to probe and feed on virus-coated leaves of the local lesion host *Nicotiana glutinosa*. Contrary to what has been claimed for four decades, aphids are apparently unable to inoculate this highly infectious virus into plants with their mouthparts.

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In earlier papers (1, 3), it was reported that aphids can inoculate plants with tobacco mosaic virus (TMV) by clawing. Presumably, virus particles on the claws, or on the leaf surface in the case of TMV-sprayed leaves, or both were inoculated into plants when they were wounded by the claws. Foliar hairs on *Nicotiana glutinosa* L. were found to serve as infection sites for TMV introduced by aphid clawing (3). The suitability of tomato as a virus source plant was attributed to glandular leaf hairs which readily contaminate the claws with TMV which can then be inoculated into virus-free *N. glutinosa* plants by clawing (1, 3).

The high numbers of inoculations which occurred in experiments on aphid clawing and the fact that in one experiment aphids with intact or nonfunctional mouthparts, respectively, were equally efficient in inoculating TMV into leaves sprayed with virus suspension (1) led us to believe "that in all published accounts [of aphid transmission of TMV] the infections could have resulted from TMV introduced by clawing, and that there is no unequivocal evidence of infections from TMV introduced by the mouthparts." Pirone has also reported data which he interprets as considerably weakening the case for inoculation of TMV by aphid stylets (10). The purpose of this research, therefore, is to revaluate the role of the mouthparts in the inoculation of TMV into plants by aphids.

MATERIALS AND METHODS.—The materials used included tobacco mosaic virus (TMV) in water at 4 mg/ml, young plants of the local lesion host *N. glutinosa*, and wingless, and in all but one case, adults of the aphids *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas). Plants in about the six-leaf stage were trimmed to the middle two leaves, allowed at least a 30-min healing period, sprayed to the dripping point with TMV suspension, and allowed to dry before being infested with aphids. Aphids were prevented from inoculating the test leaves by clawing either by using nymphs which were too small to injure plants by clawing, by amputating their claws prior to placing them on the leaves, or by gluing their feet to the leaf. In the latter case, virus suspension was applied to the leaf by placing individual droplets on the leaf surface under the aphid's mouthparts rather than by spraying it over the entire surface.

RESULTS AND DISCUSSION.—Initially, we conducted an experiment to determine what effect, if any, damaging the mouthparts of adult *M. persicae* would have on their ability to inoculate TMV into TMV-sprayed leaves. Presumably, if the mouthparts were responsible for any of the inoculations reported by previous researchers, there would be a decline in the number of infections occurring on leaves infested with aphids having nonfunctional mouthparts. Aphids with their mouthparts intact or crushed by forceps, respectively, were collected in glass vials and dropped en masse on virus-sprayed leaves at a rate of 150 aphids/leaf, and sprayed with malathion 3 hr later. Insects with crushed mouthparts inoculated the plants with TMV as often as those with intact mouthparts. Totals of 34 and 33 lesions occurred on leaves infested with 1,500 aphids having intact or nonfunctional mouthparts, respectively.

Similar results were obtained when the ability of these two aphid groups (intact mouthparts versus crushed mouthparts) to inoculate plants with TMV was again compared. This time, however, aphids were either applied to leaves one at a time or en masse at a

TABLE 1. Inoculation of tobacco mosaic virus (TMV) into *Nicotiana glutinosa* by aphids (*Myzus persicae*) with intact (+) or crushed (–) mouthparts

Mouthparts	Aphid application[a]	Inoculations (lesions/1,000 aphids tested)
+	S	28
–	S	19
+	EM	41
–	EM	38

[a] Adult, wingless *Myzus persicae* were applied to TMV-sprayed leaves of *N. glutinosa* at a rate of 50 aphids/leaf either singly (S) or en masse (EM) and sprayed with malathion 4 hr later.

rate of 50 aphids/leaf and sprayed with malathion 4 hr later. As expected, more infections resulted with both groups when aphids were dropped on leaves en masse (Table 1), a technique which resulted in greater entanglement of the aphids, more clawing, and thus more TMV infections, than when aphids are applied to leaves one at a time. Aphids with nonfunctional mouthparts that have been placed on leaves one at a time are not likely to get into many situations which would induce clawing (1), which would explain why these aphids inoculated TMV into leaves less frequently than did their counterparts handled in the same way but having intact mouthparts. The infections we obtained using aphids with nonfunctional mouthparts stands in contrast to earlier reports (6, 7, 9) that these aphids are unable to inoculate TMV into plants.

Many more infections occur on TMV-sprayed leaves which are clawed by *M. euphorbiae* than on those clawed by *M. persicae*. This is apparently a reflection of the larger aphid's ability to injure many more plant cells than the much smaller *M. persicae* (1). Hoggan (4, 5) and Teakle & Sylvester (12) also reported that a larger species of aphid inoculated TMV into plants more effectively than a smaller one. We, therefore, designed an experiment to compare the ability of various developmental instars of a single aphid species to inoculate plants with TMV. Our reasoning was that with very small nymphs, which would presumably be unable to injure plants by clawing, any infections which might result could be attributed to the mouthparts. Virus-sprayed leaves were infested with aphids previously sorted according to size into categories of large, medium, and small. The large category consisted of wingless adults of *M. persicae*, the medium of a mixture of 2nd- through 4th-instar nymphs, and the small of 1st-instar nymphs born by adults left on rape plants overnight. Plants were infested either with adults or a mixture of 2nd- through 4th-instar nymphs at a rate of 100 aphids/leaf, while a total of 15 leaves were infested with 1st-instar nymphs at from 120 to 500 aphids/leaf. As controls for background contamination, 10 leaves were sprayed with virus but not infested. All plants were left overnight and then sprayed with malathion. The ability of the aphids to inoculate TMV into plants was shown to depend entirely on their ability to injure plant cells by clawing. On leaves infested with adults, approximately 12 lesions developed for every 100 aphids used, while less than one-tenth this number developed on leaves infested with medium-sized nymphs (Table 2). Only two infections occurred on leaves infested with the 1st-instar nymphs, even though nymphs probed test leaves more often and remained on them far longer than did adult aphids. These two lesions cannot be attributed with certainty to the insects because two infections also developed on the control leaves. This is in contrast to Pirone's (9) report that the probing and feeding of 1st-instar nymphs on TMV-sprayed leaves results in lesion production.

One could argue that adult aphids probe plants differently than do nymphs and in a manner more conducive to inoculation. This hypothesis was tested in two experiments in which adult *M. persicae* were given opportunities to inoculate TMV into leaves during either brief probes, long probes, or both. Plants were trimmed to a single leaf which was sprayed with virus on the upper surface only and allowed to dry. In the first experiment, aphids which had been starved overnight in glass vials were allowed to probe the virus-coated surface. The aphids began probing almost immediately after being placed on test leaves, and their activity between probes consisted of walking across the surface. Each probe was observed under magnification and only probes of from 15 to 60 sec in duration were counted. A "map" was made of each leaf and checks were placed at points on the map where probes had occurred. Probes were limited to 1 min or less in order to exclude the possibility of the aphids' mouthparts becoming stuck in the plant tissue after long probes, a situation conducive to injury of plant cells by clawing during the aphids struggle to withdraw (1). If aphids did not terminate a probe voluntarily during the 1-min period, they were gently induced to do so by disturbing them with a brush. No lesions developed on any of 11 test leaves which were probed more than 1,000 times by a total of 67 aphids. This is in contrast to Pirone's (10) report that individual aphids inoculated leaves when they were allowed to walk for 25-30 sec on TMV-sprayed leaves.

In the second experiment, 200 adults of both *M. euphorbiae* and *M. persicae* were allowed to make both brief and long probes into test leaves; aphids were prevented from clawing by first allowing them to assume a probing position and then gluing their feet to the leaf surface with fast-drying cement (Duco). Aphids glued in this fashion readily probed in an apparently normal manner, and a determination of the depth of stylet penetration in the plant tissue showed that the aphids made both superficial and deep probes. Droplets of TMV suspension were placed on the leaf directly under the mouthparts. Aphids probed the leaves through the droplets and after the droplets had dried. Each aphid was allowed to probe for a 2-3 day period before being killed with

TABLE 2. Inoculations of tobacco mosaic virus into *Nicotiana glutinosa* by aphids (*Myzus persicae*) ranging in size from adults to 1st-instar nymphs

Aphid size	Inoculations[a] (lesions/aphids tested)
Adults	117/1,000
Mixture (2nd-4th instars)	33/3,500
1st instars	2/5,500[b]

[a] Adults and mixture of instars of *M. persicae* were applied to TMV-sprayed leaves at a rate of 100 aphids/leaf, while a total of 15 leaves were infested with 1st-instar nymphs at a rate of from 120 to 500 nymphs/leaf.

[b] Two lesions also occurred on 10 control leaves which were sprayed with virus but not infested with aphids.

malathion, and virus droplets were placed beneath the mouthparts of each insect three or more times during the first 2 days of probing. No lesions developed on any of the test leaves, indicating that none of the 400 aphids was able to inoculate TMV into plants via the mouthparts. To test whether the glue affected the susceptibility of cells to infection by TMV, 10 adults of *M. euphorbiae* were anaesthetized with carbon dioxide, glued by the labium to leaf hairs, surrounded first by droplets of glue and then by droplets of TMV suspension placed between the glue, allowed 3 hr in which to struggle free, and finally killed with malathion. Lesions developed between and around the dried glue in areas clawed by the aphids. Even cells protected by the glue from clawing injury eventually became infected with TMV when engulfed by the developing lesions.

Finally, it was found that adults of *M. persicae* with their claws amputated to prevent clawing, could not inoculate TMV into TMV-sprayed leaves by probing or feeding. Aphids were applied to the leaves en masse at a rate of 50 aphids/leaf and killed with malathion 12 hr later. No lesions developed on any of 20 virus-sprayed leaves infested with insects whose claws had been amputated. Leaves infested in the same manner with 1,000 aphids having intact claws developed a total of 119 infections even though these aphids probed and fed less often and remained on test leaves for a shorter time than did the treated insects.

Pirone (10) recently reported that individual aphids could transmit TMV simply by walking for 1 min first on a virus-sprayed source leaf and then for 1 min on a virus-free test leaf. This was reported to "considerably weaken the case for inoculation of TMV by aphid stylets." However, the claim for inoculation by walking conflicts with earlier reports by Pirone (9) and by Lojek & Orlob (6) that TMV-sprayed leaves infested with many hundreds of styletless aphids developed no infections after the aphids were allowed to walk on them for from several hr to overnight.

Lojek & Orlob (8) found that aphids can transmit TMV from tobacco plants doubly infected with TMV and cucumber mosaic virus. However, they pointed out that since their experiments did not exclude the possibility of transmission via clawing, there is a need to determine whether the claws or mouthparts or both are involved in this type of transmission. Most recently, Pirone & Shaw (11) reported that aphids can transmit to tobacco plants, via their mouthparts, poly-L-ornithine-treated TMV acquired through a stretched Parafilm membrane. Controls for transmission by clawing consisted of aphids which were allowed to walk on, but not probe, membranes containing similarly treated TMV suspensions. However, since probing aphids sometimes claw through membranes while withdrawing their mouthparts (3), more stringent controls are needed before it can be concluded that treatment with poly-L-ornithine renders TMV transmissible via aphids stylets. Tests for TMV transmission to tobacco by clawless aphids or 1st-instar nymphs previously allowed to probe membranes containing poly-L-ornithine-treated TMV suspensions should prove useful in this regard.

We conclude that aphids are not able to inoculate TMV into plants via their mouthparts. All previous reports claiming to show inoculation by this means can be explained solely on the basis of inoculation by clawing (1). Even the earlier accounts of "natural" transmission (4, 5) can be attributed to the aphids' claws becoming contaminated with virus from cells of systemically infected source plants.

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