# Mineral Oil Interferes with Retention of Tobacco Etch Potyvirus in the Stylets of *Myzus persicae*

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## ABSTRACT

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Light microscopic autoradiography was used to study the mode of action of mineral oil in reducing transmission of tobacco etch potyvirus (TEV) by *Myzus persicae*. The ability of aphids to transmit $^{125}$I-labeled TEV virions was abolished or drastically reduced by probing of mineral oil-sprayed membranes or leaves prior to virus acquisition and mineral oil-sprayed leaves after TEV acquisition. The lack of TEV transmission correlated with inability to retain labeled virions in the stylets. TEV transmission and retention of label in the stylets were closely correlated for control aphids. Our results support the hypothesis that mineral oil interferes with the retention of virions in aphid stylets.

Bradley et al. (2–4) first reported that mineral oil has an inhibitory effect on transmission of a virus transmitted by aphids in a nonpersistent manner. Since then, there have been many reports of mineral oil use in laboratory and field applications, alone and in combination with insecticides, on a wide variety of crops (1,5–7, 9,20,22–25,27,29).

The mechanism by which mineral oil prevents aphid transmission of viruses is still not understood, however. Nonpersistently transmitted viruses have a noncirculative relationship with the vector, and various lines of evidence indicate that the virions retained in the food canal of the stylets and perhaps other parts of the foregut are those involved in the transmission process (8). Bradley (2), Simons et al. (21), and Vanderveken and Dutrecq (26) reported that contacts between the labium and a mineral oil-treated leaf significantly reduced virus transmission. They concluded that the site of the inhibition was the labium and that mineral oil somehow interfered with virus attachment to aphid mouth parts. An alternative explanation was proposed by Simons et al. (21) who reported that the presence of mineral oil on leaf surfaces affected aphid behavior, as measured by electronic monitoring of aphid probing, salivation, and feeding. Other studies have concluded, however, that mineral oil did not affect aphid feeding behavior (2,11, 18,24). In a recent detailed study using electronic monitoring, Powell (16) found that mineral oil did not decrease the duration of either stylet penetration or the stylet punctures of cell membranes, which are correlated with virus transmission (15,17), and concluded that behavioral alteration did not explain the mode of action of mineral oil.

Although an effect on behavior therefore seems unlikely to account for the effect of mineral oil, thus far there has been no direct evidence to support a mode of action based on virus retention (15–17). In a recent study (28), the loss of aphid transmissibility of potyvirus mutants was highly correlated with lack of virion retention in the stylets. This provides good evidence that stylet-retained virions are involved in potyvirus transmission and makes disruption of stylet retention a logical explanation for the effect of mineral oil. In the current investigation, we traced the fate of $^{125}$I-labeled tobacco etch virus (TEV) virions in aphids to test this hypothesis.

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## MATERIALS AND METHODS

**Virion purification and iodination.** The highly aphid-transmissible strain of TEV was a strain used in previous studies (12,13). Virions were purified by the method of Murphy et al. (10). Virions were radioiodinated with Iodogen (Pierce Chemical Co., Rockford, IL) according to the manufacturer's instructions; all reactions were conducted at 4°C. Two hundred microliters of purified virions (4.2 mg/ml) was reacted for 20 min with 1 mCi of Na$^{125}$I. $^{125}$I-labeled virions were separated from unreacted Na$^{125}$I by gel filtration on Sephadex G-25 equilibrated with 20 mM Tris-HCl buffer containing 1 mM EDTA, pH 7.5. The concentration of labeled virions was determined spectrophotometrically. Specific label intensity was 4,754 dpm/ng of virions as determined in a single-well gamma counter (Bioscan, Washington DC).

**Application of mineral oil.** JMS stylet oil (JMS Flower Farms, Vero Beach, FL) was used at a concentration of 0.75% (vol/vol) and emulsified in water. The emulsion was sprayed onto plants or membranes with a hand-held sprayer, with compressed air (~20 psi) as the propellant. The film was allowed to dry, unless otherwise noted, before tests were conducted. Because the oil emulsion causes rupture and dissolution of the Parafilm membranes normally used for aphid acquisition of purified virions, non–oil-soluble membranes were prepared by stretching wastebin liner bags made of low density resin (product GR24L, Waverly Plastics Co., Waverly, IA) to a thickness similar to that of stretched Parafilm membranes.

**TEV acquisition and transmission by aphids.** *Myzus persicae* (Sulzer) were reared and handled as previously described (19). Apterae were collected and kept in glass vials for 2 to 3 h of preacquisition fasting. The acquisition mixture contained 100 to 200 µg of $^{125}$I-labeled TEV virions per ml and an amount of partially purified potato virus Y helper component sufficient to allow a maximum level of transmission. General procedures for membrane acquisition are described elsewhere (12,14). About 30 aphids were placed in a feeding chamber for a 10-min acquisition access period. Only those aphids that were still on the membrane at the end

of the acquisition access period were selected for further processing. Variations of this procedure involved allowing aphids to make brief (<1 min) observed probes on oil-sprayed or control leaves before or after membrane acquisition. These variations are described in the results. For transmission tests, one aphid was placed on each tobacco (*Nicotiana tabacum* L. 'Burley 21') seedling test plant. Aphids were allowed to remain on test plants overnight (14 to 18 h) unless otherwise stated, after which plants were sprayed with an insecticide and placed in a growth room for symptom development.

**Autoradiography of aphid stylets.** Aphids were placed in polyethylene specimen-processing holders that were 14 mm in diameter and 18 mm high and perforated at the bottom (Electron Microscopy Sciences, Fort Washington, PA) and were immediately dipped in liquid nitrogen. Aphids were thawed at room temperature and transferred onto a piece of double-stick tape on a glass slide (20 to 30 aphids per slide) oriented ventral side up. The stylets of each aphid were carefully separated from the stylet groove of the proboscis and laid flat on the tape with a sharpened insect pin. The slides were coated with liquid nuclear track emulsion (Type NTB2, Eastman-Kodak Co., Rochester, NY) according to the manufacture's instructions, dried thoroughly, and stored at 4°C in the dark for 4 weeks. Based on preliminary trials, a 4-week storage period gave satisfactory results for TEV virion concentrations ranging from 10 to 500 µg/ml. After storage, slides were developed in the dark room following the manufacturer's instructions. Slides were examined with a Zeiss photomicroscope III (Thornwood, NY).

**Liquid scintillation counting for TEV virion uptake by aphids.** About 30 apterous fourth- or fifth-instar aphids of uniform size were placed in each feeding chamber for 10-min acquisition access to the labeled virion-helper component mixture. Ten aphids still probing the membrane at the end of the acquisition access period were grouped and transferred to a liquid scintillation counting vial. They were thoroughly crushed with a wooden applicator stick; the part of the stick that touched the aphids was broken off and left in the vial. $H_2O$ (0.5 ml) and counting cocktail (4.5 ml) (Bio-Safe II, Research Products International Co., Mount Prospect, IL) were added and mixed. Radioactivity was measured in a liquid scintillation counter (TRI-CARB liquid scintillation analyzer, model 2200CA, Packard Instrument Co., Downers Grove, IL). Aphids that acquired an unlabeled virion-helper component mixture were used as background controls. Six vials were counted for each treatment, and experiments were repeated twice.

Data were subjected to analysis of variance, and means were separated by Duncan's new multiple range test (SAS Institute, Cary, NC).

TABLE 1. Effect of acquisition access through mineral oil-covered membranes on tobacco etch virus (TEV) transmission and retention of labeled TEV virions in aphid stylets[x]

| Membrane | Autoradiography | | Transmission | |
|---|---|---|---|---|
| | Ratio[y] | Percent | Ratio[z] | Percent |
| Oil-sprayed plastic | | | | |
| Labeled TEV virions | 3/308 | 0.97 | 0/50 | 0 |
| Unlabeled TEV virions | ... | ... | 0/40 | 0 |
| Untreated plastic | | | | |
| Labeled TEV virions | 41/176 | 23.29 | 12/40 | 30 |
| Unlabeled TEV virions | ... | ... | 15/40 | 37.5 |
| Parafilm | | | | |
| Labeled TEV virions | 78/144 | 54.17 | 18/40 | 45 |
| Unlabeled TEV virions | 0/40 | 0 | 26/40 | 65 |

[x] Pooled results of two experiments.
[y] Number of aphids with label in stylets/number of aphids examined.
[z] Number of plants infected/ number of plants tested. A single aphid was placed on each test plant.

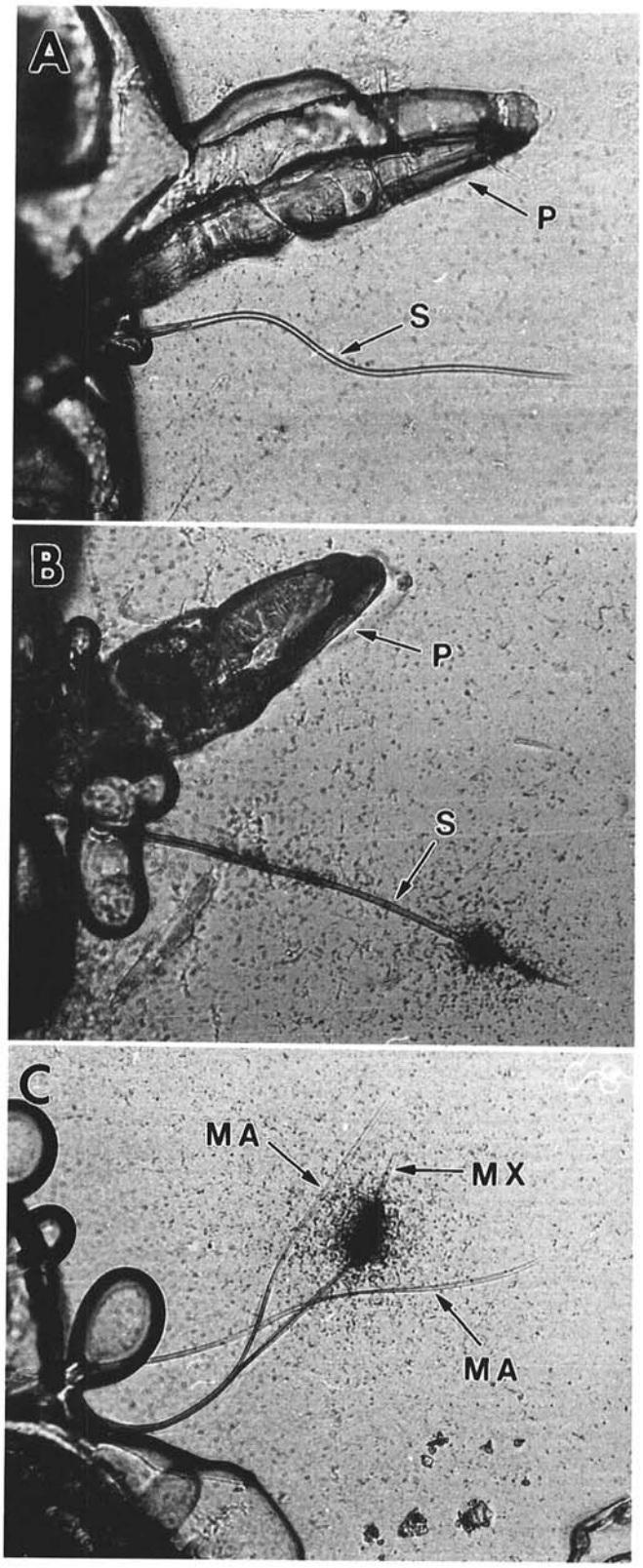


**Fig. 1.** Autoradiographs of stylets of *Myzus persicae* given acquisition access to $^{125}$I-labeled tobacco etch virus virions. **A,** Stylets typical of those in which no $^{125}$I-label was detected (Tables 1, 3, and 4). **B,** Stylets of an aphid that acquired labeled virus through a plastic membrane with no oil application; similar labeling was present in other stylets in which $^{125}$I-label was detected (Tables 1, 3, and 4), although the precise distribution of label could differ. **C,** Distribution of label in stylets that have separated, showing label associated only with the food canal formed by the maxillary stylets. MA = mandibular stylets; MX = maxillary stylets; P = proboscis; S = stylet. 420× magnification.

TABLE 2. Comparison of the amount of $^{125}$I-labeled tobacco etch virus (TEV) virions in the bodies of aphids given acquisition access through different feeding membranes

| Membrane | CPM[z] |
|---|---|
| Parafilm | 87.63 ± 3.9 a |
| Oil-sprayed plastic | 67.85 ± 6.2 b |
| Untreated plastic | 71.21 ± 4.7 b |
| Parafilm with unlabeled TEV virions | 37.47 ± 2.3 c |

[z] CPM = counts per minute. Determined by liquid scintillation counting of crushed aphids. Values are means ± standard errors from two trials, each with six replicates. Numbers followed by the same letter are not significantly different at $P = 0.01$.

TABLE 3. Effect of probing mineral oil-sprayed leaves prior to membrane acquisition of tobacco etch virus virions on virus transmission and retention in stylets[x]

| Aphids probed on | Transmission[y] | | Label in stylets[z] | |
|---|---|---|---|---|
| | Ratio | Percent | Ratio | Percent |
| Oil-sprayed plant | 0/120 | 0 | 0/92 | 0 |
| Control plant | 20/120 | 16.67 | 11/88 | 12.50 |

[x] Pooled results of two experiments.
[y] Ratio: number of plants infected/total number of test plants. A single aphid was placed on each test plant.
[z] Determined by autoradiography. Ratio: number of aphids positive/total number examined.

## RESULTS

**Effect of probing through mineral oil-covered membranes.** Aphids allowed acquisition access through plastic membranes to which oil had been applied were compared with controls that probed untreated plastic or Parafilm membranes. After acquisition access, aphids from each treatment were either placed on test plants or processed for autoradiography. Transmission tests and autoradiography were done with unlabeled TEV virions as additional controls. Aphids that made acquisition probes through oil did not transmit TEV, and label was found in less than 1% of the stylets (Table 1). There was a close correlation between transmission and stylet retention for the other treatments. Labeling had a slight effect on transmission (Table 1). There was no noticeable difference in label intensity or distribution in the stylets due to different membranes (Fig. 1).

**Comparative uptake of $^{125}$I-labeled virions by aphids.** To determine whether the differences in stylet retention were due to differences in overall virion uptake, aphids given acquisition access were processed for liquid scintillation counting. There was no significant difference in the amount of radioactivity in aphids that acquired TEV virions through oil-covered and untreated plastic (Table 2). Aphids that acquired TEV virions through Parafilm contained more label, which probably is reflected in their greater tendency to transmit (Table 1).

**Effect of preacquisition probing of oil-sprayed leaves.** Starved aphids were allowed to probe on oil-sprayed or control tobacco leaves. They were then transferred to Parafilm-membrane feeding chambers for 10-min acquisition access, after which individual aphids were either placed on test plants or processed for autoradiography. Aphids that had probed oil-treated leaves prior to acquisition did not transmit TEV nor did they retain label in the stylets (Table 3). Again there was a close correlation between transmission and stylet retention for the controls, although the overall levels were low (Tables 1 and 4), probably due to loss of the starvation effect.

**Effect of postacquisition probing of mineral oil-sprayed leaves.** Aphids were given 10-min acquisition access to labeled TEV virions through Parafilm membranes. They were then allowed to probe oil-sprayed or control leaves before transfer to test plants or processing for autoradiography. Additional controls were assayed directly after removal from the feeding membrane. Transmission and stylet retention were both dramatically reduced by prior probing on oil-sprayed leaves; probing an unsprayed leaf also resulted in a lesser reduction compared to aphids tested directly after acquisition (Table 4).

TABLE 4. Effect of postacquisition probing of mineral oil-sprayed leaves on tobacco etch virus (TEV) transmission and retention of labeled TEV virions in aphid stylets[x]

| Postacquisition probe on | Transmission[y] | | Label in stylets[z] | |
|---|---|---|---|---|
| | Ratio | Percent | Ratio | Percent |
| Oil-sprayed plant | 1/60 | 1.67 | 6/131 | 4.58 |
| Control plant | 11/60 | 18.30 | 28/123 | 22.76 |
| No probe | 31/60 | 51.60 | 53/109 | 61.47 |

[x] Pooled results of two experiments.
[y] Ratio: number of plants infected/total number of test plants. A single aphid was placed on each test plant.
[z] Determined by autoradiography. Ratio: number of aphids positive/total number examined.

## DISCUSSION

Our results show a close correlation between the decrease in aphid transmission of TEV and nonretention of $^{125}$I-labeled TEV virions in the stylets and, thus, provide direct evidence supporting the hypothesis that mineral oil acts by interfering with virus retention (2,21,25). In our previous study (28), retention of label in the stylets was correlated with retention of virions in the food canal, as determined by transmission electron microscopy of thin-sectioned stylets, and hence, it is reasonable to assume that we are also detecting virion retention in the current study. Transmission electron microscope examination of thin-sectioned stylets (28) and autoradiography of stylet bundles that have separated (28; Fig. 1) show that the site of retention is the food canal formed by the maxillary stylets. We speculate that during stylet penetration of the oil film a hydrophobic layer of oil forms in the food canal, which hinders virus retention.

Both pre- and postacquisition probing through oil affect virus transmission and stylet retention. With preacquisition probing, it seems clear that mineral oil acts by preventing virions from being retained in the stylets. Postacquisition probing of oil-treated surfaces evidently dislodges virions from the stylet. Our data do not allow determination of whether this results in virions being displaced in the direction of the gut (and, thus, being unavailable for transmission) or being egested into the plant (in which case the observed decrease in transmission would be due to an effect of oil on the virus or the cell).

Both our current and previous studies (28) indicate the importance of virus retention in the food canal to the transmission process. This suggests that increased emphasis on the development of control chemicals or strategies that interfere with this process would be justified.

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