Use of Acetone to Facilitate Aphid Harvesting for Plant Virus Transmission Assays

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ABSTRACT

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Acetone as an aphid anesthetic was compared with mechanical removal of aphids from leaves to enhance the harvesting and handling of large populations of the aphid *Myzus persicae*. The effect of these two procedures on aphid virus transmission efficiency was assessed with a persistent and a nonpersistent virus using potato leafroll virus (PLRV) and papaya ringspot virus type W (PRSV-W), respectively. The use of acetone was 8–10 times faster and about 30% more efficient than the camel's-hair brush technique for harvesting *M. persicae* from leaves of turnip, *Malva parviflora*, and sweet pepper. Even small nymphs could be collected and handled easily, indicating an additional benefit of the acetone method. There were no significant differences in transmission efficiency of aphids harvested via acetone or the camel's-hair brush; rates were 60 and 54%, respectively, with PLRV and 95 and 93%, respectively, with PRSV-W. The acetone procedure was useful for handling *M. persicae* and possibly other aphid virus vectors also.

Handling aphids for plant virus transmission assays can be time-consuming and laborious because care must be taken to avoid damaging aphid stylets during removal of aphids from plants. Handling is particularly important for nymphs because they are the least suitable for manipulations (7), yet they are effective vectors (8). Stylet damage may result in failure of aphids to penetrate leaves (4) and, therefore, limit transmissions with either persistently or nonpersistently transmitted plant viruses (2).

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Different procedures have been suggested to be effective for transferring aphids from plants without damaging aphid mouthparts. These include: 1) allowing natural or artificial (petiole vacuum pumping) wilting of infected leaves laid on the target plant (1); 2) placing infested tissue into a petri dish and allowing it to wilt under the warmth of a strong light bulb for 40–60 min (1); 3) gently tapping detached leaves over a white smooth background (plastic or paper) tray to cause aphids that are not feeding to drop off and be collected into a vial (11); and 4) stimulating aphids to stop feeding and start wandering by gently tickling their antennae or breathing on them before picking them up with the wet tip of a fine camel's-hair brush (12). None of these methods is efficient for handling large aphid populations when time for virus acquisition and/or inoculation must be controlled.

A method that causes feeding aphids to withdraw their stylets and to fall from leaves would be ideal for virus transmission purposes. Using a canister containing a pad soaked with methyl isobutyl ketone, Gray and Schuh (5) developed a sampling method that facilitated the collection of pea aphids (*Macrosiphum pisi* Kaltenbach) from plant tips under field conditions. They showed that even first instar aphids withdrew their stylets and dropped from plants in less than 5 min. All aphids were anesthetized in

about 10 min but recovered when removed from the canister. Aphids can also be anesthetized by short exposure to a CO_2 stream, but this can reduce stylet penetration and probing frequency, resulting in less efficient transmission of nonpersistent viruses (4,10).

In this study we compared the efficiency of acetone as an anesthetic and the camel's-hair brush technique to handle and harvest apterous *Myzus persicae* (Sulzer) for plant virus transmission assays. The effect of the acetone on the efficiency of *M. persicae* to transmit a persistent and a nonpersistent virus was also evaluated by working with potato leafroll virus (PLRV) and papaya ringspot virus type W (PRSV-W), respectively.

MATERIALS AND METHODS

Plants and growing conditions. *Malva parviflora* L., *Physalis* sp. (3), potato (*Solanum tuberosum* L. 'Russet Burbank'), sweet pepper (*Capsicum annuum* L. 'Casca Dura Ikeda'), turnip (*Brassica rapa* L.), and zucchini squash (*Cucurbita pepo* L. 'Caserta') were grown in a soil mix in 10-cm-diameter pots. Plants were maintained under greenhouse conditions as described below.

Viruses. An isolate of PLRV (persistent transmission), characterized by Sibara (9) as a common strain from Wisconsin, and a common Brazilian strain of PRSV-W (nonpersistent transmission) were used in the experiments. PLRV was maintained in potato cv. Russet Burbank, and PRSV-W was propagated in zucchini squash cv. Caserta.

Aphid harvesting with acetone. In experiments with PLRV, done at the University of Wisconsin-Madison, virus-free *M. persicae* were reared on turnip plants confined in insect cages at greenhouse temperatures of 20–24 C, under 5,000 lx (fluorescent light) with a daily photoperiod of 16 hr, as suggested by MacGillivray (6). Apterous *M. persicae* were harvested from turnip leaves as follows: 1) 100 μl of acetone was placed in a glass petri dish (15 × 2 cm) at room temperature; 2) a single wipe tissue was placed over the dish, and plant leaves infested with aphids were placed on the top of the tissue; 3) the dish was covered with the lid for 2–3 min; 4) plant leaves and wipe tissue were gently tapped over a smooth white plastic sheet with a camel's-hair brush; 5) anesthetized aphids were funneled from the plastic sheet into a glass vial, which was covered with Parafilm M; and 6) aphids were allowed a few minutes to recover from the effects of the acetone before virus transmission tests were conducted.

The experiments with PRSV-W were carried out at the Instituto Agronomico, Campinas, SP, Brazil. Virus-free *M. periscae* were reared on *M. parviflora* or sweet pepper while confined in insect cages maintained at greenhouse temperatures of 28–32 C. Apterous *M. periscae* were harvested from infested leaves as follows: 1) 200, 400, or 800 μl of acetone was placed on the bottom of 650-ml glass bottles; 2) aphid-infested leaves were glued by the petiole to the inside of the bottle lid (one leaf per lid); 3) lids were screwed onto the bottles, allowing the leaves to hang inside; 4) infested leaves were held for 4 min in bottles containing 200 μl of acetone and for 2.5 min in bottles containing 400 or 800 μl of acetone; 5) leaves were gently tapped over a plastic box to release anesthetized aphids (anesthetized aphids that had fallen inside the bottle were also transferred to the plastic box); and 6) aphids were allowed a few minutes to recover before virus transmission assays were conducted.

Aphid harvesting with camel's-hair brush. Leaves of similar size and infested with apterous *M. persicae* were selected. A moistened camel's-hair brush was used to disturb feeding aphids by gently touching their antennae, inducing them to stop feeding and start moving (12). Moving aphids were released from leaves by gently tapping the leaves over a white plastic sheet. Aphids were then funneled into glass vials, which were sealed with Parafilm M.

Testing the effect of the aphid harvesting procedure on the transmission of PLRV. Bottom leaflets from five comparable (50- to 60-day-old) potato cv. Russet Burbank plants showing typical secondary PLRV symptoms were detached and placed in petri dishes containing moistened paper towels. Immediately after harvest and recovery, aphids harvested by the acetone or camel's-hair brush procedure were transferred separately to infected leaflets (50 aphids per leaflet) for virus acquisition. Petri dishes were closed and placed inside zippered plastic bags. Aphids were allowed an acquisition access period of 48 hr at 20 C and then were removed from leaflets with a camel's-hair brush. Aphids were transferred individually to 10 healthy test plants of *Physalis* sp. (one aphid per plant) and given an inoculation access period of 3 days, after which plants were sprayed with an insecticide to kill aphids. Virus-free aphids transferred to healthy test plants served as controls. Test plants were scored visually for PLRV symptoms at intervals of 3–5 days for 30–40 days after inoculation. This experiment was repeated five times.

Testing the effect of the aphid harvesting procedure on the transmission of PRSV-W. Two independent experiments were carried out to test the effect of the aphid harvesting procedure on transmission of PRSV-W. In each experiment, 10 plants of zucchini squash cv. Caserta were used for each dose of the acetone method and the camel's-hair brush procedure. Apterous *M. persicae* harvested with the acetone and camel's-hair brush procedures were starved for 1 hr in plastic boxes. Aphids were then transferred to detached young leaves of zucchini squash cv. Caserta systemically infected with PRSV-W. After an acquisition access period of 15 min, aphids were transferred individually to healthy plants of zucchini squash cv. Caserta (10 aphids per plant) for virus transmission tests. Virus-free aphids were used as controls. After 24 hr, plants were sprayed with an insecticide to kill aphids. Test plants were maintained under greenhouse conditions for 3 wk to record the development of symptoms.

RESULTS AND DISCUSSION

Comparison of acetone and camel's-hair brush procedures for harvesting *M. persicae*. The results of three experiments in which the efficiency of acetone and camel's-hair brush procedures for harvesting *M. persicae* from turnip leaves were compared (Table 1). An average of 94% of the aphids was harvested with the acetone procedure, compared with 61% harvested with the camel's-hair brush method. After aphids were allowed to recover in glass vials for 1–1.5 hr, the percentages of viable aphids recovered from the vials were 86 and 92% for the acetone and camel's-hair brush procedures, respectively.

The acetone method was always faster than the camel's-hair brush procedure for harvesting *M. persicae* from turnip leaves. When the acetone procedure was used, practically all aphids were harvested from five turnip leaves in 4–5 min, whereas with the camel's-hair brush method, 8–9 min were needed to harvest

Table 1. Number and percentage of *Myzus persicae* collected from turnip leaves by means of the acetone (AC) and camel's-hair brush (CHB) procedures

	Initial aphid population[x]		Collected from leaves				Recovered from vials[y]			
			No.		%		No.		%	
Experiment	AC	CHB	AC	CHB	AC	CHB	AC	CHB	AC	CHB
1	153	143	144	92	94	64	116	83	81	90
2	137	112	127	63	93	56	105	62	82	99
3	173	188	166	117	95	62	157	100	95	86
Mean	154 a[z]	148 a	145 a	91 b	94	61	126 a	82 a	86	92

[x] Values are mean of five turnip leaves.
[y] Counted at the top and on the walls of the vials 1–1.5 hr after collection.
[z] Mean values with the same letters do not differ significantly ($\alpha = 0.05$).

Table 2. Effect of the acetone (AC) and the camel's-hair brush (CHB) procedures on the efficiency of *Myzus persicae* to transmit potato leafroll virus to *Physalis* sp.

Experiment	No. of plants infected/ no. of plants inoculated[z]	
	AC	CHB
1	8/10	6/10
2	4/10	4/10
3	5/10	4/10
4	8/10	6/10
5	5/10	7/10
Total	30/50	27/50

[z] Numbers of infected plants were not significantly different between treatments at $P = 0.05$ (paired t test).

30 aphids (*data not shown*). Similar time differences were observed when *M. persicae* were harvested from leaves of *M. parviflora* and sweet pepper by means of these two procedures.

Although nymphs and adults were not counted separately in these experiments, more nymphs were invariably collected from infested leaves by the acetone procedure than by the camel's-hair brush method. Nymphs are more difficult than adults to transfer with a camel's-hair brush, do not withstand handling as well, and die sooner (7). Because *M. persicae* nymphs can be as efficient virus vectors as adults, efficient collection of nymphs is also important.

When the dose of acetone was increased above 100 µl per petri dish in PLRV experiments, fewer *M. persicae* were recovered. However, no effect on aphid survival was observed with increasing doses of acetone in PRSV-W experiments. In the latter case, aphids were partially anesthetized and recovered within a few minutes after being transferred to the plastic box.

Effect of the aphid harvesting procedure on transmission of PLRV. Data indicated that the efficiency of *M. persicae* to transmit PLRV was similar when aphids were collected by either method (Table 2). Of the inoculated *Physalis* sp. plants, 60% showed symptoms of stunting and leaf epinasty 30–35 days after exposure to viruliferous *M. persicae* harvested by the acetone procedure, compared with 54% of the plants exposed to viruliferous aphids harvested by the camel's-hair brush method. This difference was not statistically significant ($P > 0.05$).

Table 3. Transmission of papaya ringspot virus type W by *Myzus persicae* collected by the acetone and camel's-hair brush procedures from leaves of *Malva parviflora* and sweet pepper cv. Casca Dura Ikeda

Plant source for aphids	No. of plants infected/no. of plants inoculated[z]			
	Acetone/650-ml bottle			Camel's-hair brush
	200 µl	400 µl	800 µl	
Malva parviflora	19/20	20/20	20/20	17/20
Sweet pepper *(Capsicum annuum)*	19/20	20/20	16/20	20/20

[z] Total of two independent experiments. Numbers of infected plants were not significantly different among treatments at $P = 0.05$ (paired t test).

Effect of the aphid harvesting procedure on the transmission of PRSV-W. Results from two experiments showed that PRSV-W was efficiently transmitted by *M. persicae*, regardless of the procedure used to harvest aphids (Table 3). Although aphids were not killed at any of the acetone levels used, as shown by the transmission data, we now use 400 µl of acetone per 650-ml bottle for routine experiments. The host plant on which *M. persicae* was reared did not appear to affect the harvesting procedure or virus transmission efficiencies. Moreover, because acetone did not affect the efficiency of *M. persicae* to transmit either PLRV or PRSV-W and because nymphs can be harvested efficiently, this method appears to be effective for studies requiring large numbers of aphid adults or nymphs.

Because a camel's-hair brush was used for all experiments to remove aphids from virus source plants in transfers to test plants, we cannot state that an acetone treatment at this step would not affect transmission results. However, we do not anticipate any treatment effects. We suggest that this protocol could also be appropriate for use with other aphid species.

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