Transmission of Pigeon pea sterility mosaic virus by the Eriophyid Mite, *Aceria cajani* (Acari: Arthropoda)

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ABSTRACT

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The transmission characteristics of Pigeon pea sterility mosaic virus (PPSMV) to pigeon pea (*Cajanus cajan*) by its eriophyid mite vector, *Aceria cajani,* were studied. Nonviruliferous *A. cajani* colonies were established on detached healthy leaflets of a PPSMV-immune pigeon pea cultivar floating on water. The transmission efficiency of single *A. cajani* was up to 53% but was 100% when >5 mites per plant were used. *A. cajani* acquired PPSMV after a minimum acquisition access period (AAP) of 15 min and inoculated virus after a minimum inoculation access period (IAP) of 90 min. No latent period was observed. Starvation of *A. cajani* prior to, or following, PPSMV acquisition reduced the minimum AAP and IAP periods to 10 min and 60 min, respectively, and mites retained virus for up to 13 h. None of the mites that developed from eggs taken from PPSMV-infected leaves transmitted the virus, indicating that it is not transmitted transovarially. Taken together, these data suggest a semipersistent mode of transmission of PPSMV by *A. cajani*.

Additional keywords: sterility mosaic disease, virus transmission

Sterility mosaic disease (SMD) is the most damaging disease of pigeon pea (*Cajanus cajan*) in the Indian subcontinent. This disease, first described in 1931 from Bihar state, India (4), is endemic in all the pigeon pea–growing states of India, Bangladesh, Nepal, and Myanmar. SMD-affected plants show characteristic mosaic symptoms on leaves with reduced or no flowering (sterility), and yield reduction can be up to 90% (6). The causal agent of SMD has remained unidentified for several decades (28) but has recently been identified as a distinct virus, named Pigeon pea sterility mosaic virus (PPSMV) (9). Purified preparations of PPSMV contain slender, highly flexuous filamentous virus-like particles (VLPs) of 3 to 10 nm in diameter, a major polypeptide of 32 kDa, and five to six major RNA species of c. 1.1 to 3.5 kb (9). Polyclonal antiserum produced to purified VLP preparations reacted specifically with the 32-kDa protein in enzyme-linked immunosorbent assay (ELISA) and Western immunoblotting. Virus-infected cells contain quasi-spherical, membrane bound bodies (MBBs) of 100 to 150 nm that were labeled specifically by antibodies to the 32-kDa protein (7). Purified PPSMV preparations were not infective to plants. However, PPSMV in fresh leaf sap extracts from SMD-affected pigeon pea was transmitted experimentally by mechanical inoculation to *Nicotiana benthamiana*, but not to pigeon pea (10). Although the taxonomic relationship of PPSMV to other viruses is as yet unclear, the morphology of its VLPs in purified preparations, the number and sizes of its apparent nucleoprotein components, and the cytopathology of infected tissues show close similarities to High Plains virus (HPV) (1,7,12). However, the two viruses are serologically unrelated and differ in host range and in the vector mite species involved in transmission.

PPSMV is transmitted by the eriophyid mite, *Aceria cajani* Channabasavanna (Acari: Arthropoda) (4,27). Analysis of mite populations obtained from SMD-affected plants from several locations in India, Nepal, and Myanmar has indicated that *A. cajani* transmits PPSMV at all of these locations (8). This mite is highly host-specific with a very narrow host range, confined mainly to pigeon pea and its wild relatives, *Cajanus scarabaeoides* and *C. cajanifolius*. Adult *A. cajani* measure 200 to 250 µm and have a very short life cycle of about 2 weeks comprising egg (30 × 40 µm) and two nymphal stages (17). Mites inhabit the lower surface of leaflets and are found predominantly on symptomatic leaves of PPSMV-infected plants (8,14,25). Their feeding causes no obvious damage to the host. Once established on PPSMV-susceptible genotypes, mites can multiply to high densities within a few weeks. Their dispersal is passive, assisted mainly by wind currents.

Little is known about the mechanism of transmission of PPSMV by *A. cajani*. One study suggested that PPSMV was not transmitted transovarially by *A. cajani* but was transmitted in a persistent manner provided mites continue to feed on susceptible plants (24). Studies on mite transmission have been hindered by difficulties in rearing healthy mite colonies, manipulating individual *A. cajani,* and the lack of information on the causal agent of SMD and its unequivocal detection in plants. However, most of these difficulties have now been overcome, and this paper presents data to show that the transmission of PPSMV by *A. cajani* is probably in a semipersistent manner.

MATERIALS AND METHODS

PPSMV culture and indicator plants. The virus was maintained on the pigeon pea cv. ICP8863, which is highly susceptible to PPSMV by the leaf stapling method (15). Plants were grown in growth chambers maintained at 28°C for a 14-h day period and at 20°C for a 10-h night period, with a constant 70% relative humidity. Mite-free PPSMV-infected pigeon pea cultures were established by leaflet grafting (23) and were maintained in a separate growth chamber operating under the same environmental conditions as those given above. These graft-inoculated plants were used as PPSMV sources for experiments using nonviruliferous mite colonies. Pigeon pea cv. ICP8863 plants, 12 to 15 days old (two-leaf stage), were used as indicator test plants for all mite transmission experiments because this cultivar shows prominent mosaic symptoms to PPSMV infection 2 weeks postinoculation (pi). All experimental plants were covered individually with mite-proof cages and maintained in separate growth chambers. The cages were made from rigid PVC with the single vent holes on the top and on two

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sides of the cage sealed with a double layer of muslin. Healthy cv. ICP8863 seedlings were maintained in growth chambers used for maintaining experimental plants to detect any possible contamination by viruliferous *A. cajani*; none of these plants were found infected with PPSMV in these studies.

Detection of PPSMV infection. All experimental plants were assayed for PPSMV symptoms and by double-antibody sandwich (DAS)-ELISA using anti-PPSMV rabbit polyclonal antibodies as described previously (11). Leaf tissue (c. 100 mg) was extracted in 1 ml phosphate buffered saline containing 2% polyvinyl pyrrolidone (molecular weight 40,000), 0.2% ovalbumin, and 0.02% Tween-20, and 100 µl of this extract was placed in wells of ELISA plates precoated with PPSMV immuno-γ-globulins (IgGs) at 1 µg ml^{-1}. Penicillinase (PNC)-labeled PPSMV IgGs were used at 1:1,000 dilution to detect trapped antigen. Sodium penicillin G was used at 0.05 mg ml^{-1} in substrate buffer (0.015% [wt/vol] bromothymol blue in 5 mM NaOH, pH 7.2), and readings were made at 620 nm in a Multiscan Plus ELISA plate reader (Labsystems, Helsinki, Finland). Absorbance readings at 620 nm of three or more times those of healthy plant extracts were considered PPSMV positive.

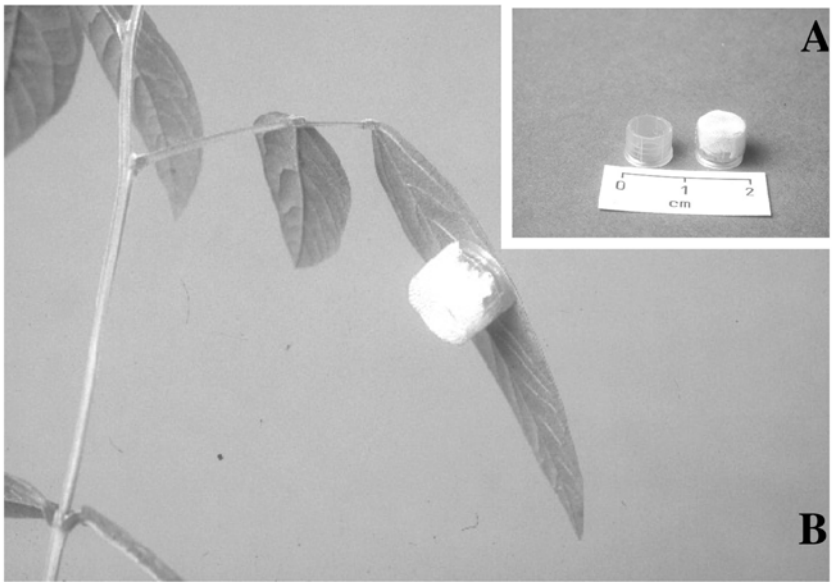

Fig. 1. A, Polypropylene micro-cages used for containing single *Aceria cajani* on leaflets. **B,** Micro-cage attached to a pigeon pea leaflet.

Table 1. Transmission efficiency to seedlings of pigeon pea cv. ICP 8863 of Pigeon pea sterility mosaic virus by different numbers of *Aceria cajani*

	No. plants infected/no. plants tested (% transmission)			
No. *A. cajani*/plant	**Exp. 1**	**Exp. 2**	**Exp. 3**	**Mean %**
1	8/20 (40)	7/20 (35)	9/20 (45)	40
2	7/15 (46)	10/15 (66)	7/15 (46)	53
3	11/15 (73)	9/15 (60)	8/10 (80)	70
4	7/10 (70)	8/10 (80)	6/10 (60)	70
5	10/10 (100)	8/10 (80)	8/10 (80)	87
10	9/10 (90)	10/10 (100)	10/10 (100)	97
20	10/10 (100)	10/10 (100)	10/10 (100)	100

Table 2. Transmission to pigeon pea cv. ICP8863 seedlings of Pigeon pea sterility mosaic virus (PPSMV) by groups of 10 *Aceria cajani* reared on PPSMV-infected pigeon pea and given different inoculation access periods (IAP)

	No. plants infected/no. plants tested (% transmission)			
IAP (h)[a]	**Exp. 1**	**Exp. 2**	**Exp. 3**	**Mean %**
0.5	0/10 (0)	0/10 (0)	0/10 (0)	0
1	0/10 (0)	0/10 (0)	0/10 (0)	0
1.5	1/10 (10)	0/10 (0)	1/10 (10)	7
2	2/10 (20)	2/10 (20)	1/10 (10)	17
3	3/10 (30)	2/10 (20)	2/10 (20)	23
5	8/10 (80)	8/10 (80)	6/10 (60)	73
10	9/10 (90)	10/10 (100)	10/10 (100)	97
15	10/10 (100)	10/10 (100)	nt[b]	100
24	10/10 (100)	10/10 (100)	nt	100

[a] At the end of the IAP, mite feeding was terminated by spraying test plants with contact acaricide.
[b] nt = not tested.

Handling of *A. cajani.* All manipulations of mites were done under a stereo binocular microscope. A human eyelash affixed to a 6-cm-long, 2-mm-wide wooden stick was used for transferring mites from the source plant/leaflet onto test plants. In all experiments using single mites, polypropylene micro-cages were used to confine the single mite to an area on the leaf surface for easy location of mites (Fig. 1). These micro-cages (5 × 10 mm) were made by cutting off the tip-end of 1-ml micropipette tips (Finntips, Cat. # FB56271, Fisher Scientific, Loughborough, UK) and covering one end of the residual base with a single layer of muslin cloth (Fig. 1). Micro-cages were fixed to the pigeon pea leaf surface by applying a synthetic adhesive (Vami gum, Vam Organics Ltd., New Delhi, India) on the rim at the open end. This adhesive did not damage the leaf surface or have any noticeable toxic effect on mites, and allowed easy removal of micro-cages for multiple use.

Generation of healthy mites. To obtain nonviruliferous *A. cajani,* the floating leaflet technique was used. Leaflets from pigeon pea cv. ICP8136, which supports mite multiplication but is immune to PPSMV, were floated on sterile distilled water in a petri dish. Mites from PPSMV-infected pigeon pea leaves were transferred onto the floating leaflet and, after 2 days, to another healthy cv. ICP8136 floating leaflet. After a further day, groups of about 50 mites were transferred onto pigeon pea cv. ICP8863 to ensure that the mite colony was free from PPSMV. Indicator plants were caged and assayed for PPSMV at 20 to 22 pi and again at 45 to 50 days pi.

Transmission efficiency. To determine the numbers of mites required to obtain 100% virus transmission, naturally viruliferous *A. cajani* obtained from PPSMV-infected pigeon pea plants were transferred onto healthy pigeon pea cv. ICP8863 seedlings, either singly or in groups of 2, 3, 4, 5, 10, and 20 (Table 1). These plants were then caged and assayed for PPSMV 3 weeks pi.

Inoculation access period (IAP). Groups of 10 *A. cajani* from PPSMV-infected plants were transferred to individual healthy pigeon pea cv. ICP8863 seedlings and allowed different IAPs ranging from 0.5 to 24 h (Table 2). Feeding of mites was terminated by spraying plants with a contact acaricide, Kelthane (active ingredient Dicofol used at working concentration of 0.2% [vol/vol]; Indofil Chemicals Company Ltd., Mumbai, India). Plants were caged, maintained in a mite-free growth chamber, and assayed for PPSMV 3 weeks pi.

Acquisition access period (AAP). Nonviruliferous *A. cajani* were allowed to acquire PPSMV from young pigeon pea leaflets showing prominent PPSMV symptoms taken from plants infected by graft inoculation. The leaflets were floated on water in a petri dish, and mites were transferred to them for different AAPs from 5 min to 36 h (Table 3). After the AAP, either single mites or groups of 10 mites were transferred onto healthy pigeon pea cv. ICP8863 seedlings. Experiments using groups of 10 mites were replicated three times, and one trial was made using single mites. Test plants were caged and assayed for PPSMV 3 weeks pi.

Latent period. To detect a possible latent period between virus acquisition and transmission, nonviruliferous *A. cajani* were given a 45-min AAP on PPSMV-infected pigeon pea leaves. Single mites were then transferred serially four times to healthy pigeon pea cv. ICP8863 seedlings allowing 1 h IAP in one experiment and 2 h IAP in another experiment between each transfer. Plants were caged and assayed for PPSMV 3 weeks pi.

Virus retention by *A. cajani*. To study the retention of PPSMV by *A. cajani,* nonviruliferous mites were fed on PPSMV-infected leaflets (plants) for 24 h. Single mites were then transferred serially to healthy pigeon pea seedlings for IAPs of 1 to 24 h (Table 4). Mites were serially transferred three to eight times onto healthy seedlings.

Effect of starvation of *A. cajani* on PPSMV transmission. To determine the effect of starvation prior to AAP, nonviruliferous *A. cajani* were starved for 3 to 4 h on dried healthy pigeon pea leaflets in petri dishes that contained moist cotton swabs that were not in direct contact with the leaves. They were then exposed for AAPs of 5 min to 5 h on PPSMV-infected pigeon pea leaflets. At the end of the AAP, individual mites were transferred onto healthy seedlings, and the plants were covered with mite-proof cages and assayed for PPSMV 3 weeks pi.

To determine the influence of starvation following virus acquisition, nonviruliferous mites were given 45 min AAP on PPSMV-infected leaflets and then starved for 3 to 4 h on dried healthy pigeon pea leaflets. Single mites were then transferred serially three times onto healthy pigeon pea seedlings for 30 min, 1 h, and 2 h IAPs. Plants were caged and assayed for PPSMV 3 weeks pi.

The effect of starvation on the retention of PPSMV by *A. cajani* was determined by starving viruliferous mites in separate experiments for 1 to 13 h (the maximum survival time of *A. cajani*, when starved) on dried healthy pigeon pea leaflets. At the end of the starvation period, groups of 10 mites from each treatment were transferred onto healthy pigeon pea seedlings, the test plants were caged and then assayed for PPSMV 3 weeks pi.

Survival of *A. cajani* on healthy and PPSMV-infected pigeon pea. Plants of pigeon pea cv. ICP8863 were grown in a growth chamber. Three such plants were graft inoculated with PPSMV using petioles from PPSMV-infected plants and, after the appearance of SMD symptoms, were each infested with c. 200 nonviruliferous *A. cajani*. Three ungrafted plants were also each infested with c. 200 nonviruliferous *A. cajani,* and mite numbers were counted on all six plants 2 months after infestation.

RESULTS

Generation of nonviruliferous *A. cajani* colonies. None of the pigeon pea cv. ICP8863 seedlings inoculated with *A. cajani* that had fed on healthy cv. ICP8136 leaflets developed SMD symptoms, and all were negative for PPSMV in DAS-ELISA. Therefore, the mite colonies raised by this method were free from PPSMV. Additionally, the floating pigeon pea cv. ICP8136 leaflets on which mites were allowed to feed also tested negative for PPSMV in DAS-ELISA. This method for generating nonviruliferous mites is therefore very efficient and convenient, and large numbers of PPSMV-free *A. cajani* colonies can be generated within a few days.

Efficiency of *A. cajani* in transmitting PPSMV. In three separate experiments using single mites raised on PPSMV-infected plants, only 35 to 45% (mean 40%) of the inoculated plants became infected with PPSMV. However, increases in virus transmission occurred when plants were inoculated with >2 viruliferous mites (>60%; Table 1). Usually, close to 100% virus transmission occurred when plants were exposed to >5 mites, and inoculation with 20 mites always resulted in 100% virus transmission when given IAPs of 15 h or more (Table 2). SMD symptoms appeared within 1 week pi on test plants inoculated with >10 mites, whereas those inoculated with <10 mites usually took about 10 to 14 days.

IAP. None of the plants exposed to viruliferous *A. cajani* for IAP of 1 h or less

Table 3. Effect of different acquisition access periods (AAP) on the transmission of Pigeon pea sterility mosaic virus (PPSMV) by *Aceria cajani*

	No. plants infected/no. plants tested (% transmission)[a]				
AAP[b]	Exp. 1	Exp. 2	Exp. 3	Mean %	Single mite/plant
5 min	0/7 (0)	0/5 (0)	0/5 (0)	0	0/15 (0)
10 min	0/6 (0)	0/8 (0)	0/6 (0)	0	0/15 (0)
15 min	5/6 (83)	3/5 (60)	3/6 (50)	65	2/15 (13)
30 min	4/6 (67)	5/6 (83)	4/5 (80)	76	2/15 (13)
2 h	6/7 (86)	5/5 (100)	4/6 (67)	83	2/15 (13)
5 h	5/6 (83)	6/7 (86)	5/5 (100)	89	4/15 (26)
10 h	5/6 (83)	5/5 (100)	7/7 (100)	84	4/13 (30)
15 h	6/6 (100)	6/7 (86)	6/6 (100)	95	5/15 (33)
24 h	6/6 (100)	6/6 (100)	7/7 (100)	100	8/15 (53)
36 h	7/7 (100)	nt[c]	nt	100	8/15 (53)

[a] 10 mites/plant.
[b] *A. cajani* exposed to PPSMV-infected source leaflets for the times indicated. Mite feeding was not terminated after transferring onto test plants.
[c] nt = not tested.

Table 4. Retention of Pigeon pea sterility mosaic virus by single viruliferous *Aceria cajani*

	No. plants infected/no. plants tested (% transmission)[c]							
IAP[a] (h)[b]	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	7/15 (46)	3/15 (20)	0	nt[d]	nt	nt	nt	nt
5	2/14 (14)	3/14 (21)	0	nt	nt	nt	nt	nt
6	2/15 (13)	2/15 (13)	0	nt	nt	nt	nt	nt
10	3/15 (20)	0	nt	nt	nt	nt	nt	nt
15	2/11 (18)	0	nt	nt	nt	nt	nt	nt
24	2/12 (16)	0	0	0	0	0	0	0

[a] IAP = inoculation access period.
[b] Times of mite feeding on test plants before transferring to another healthy pigeon pea cv. ICP8863 seedling.
[c] Serial transfers.
[d] nt = not tested.

were infected (Table 2). Increased virus transmission occurred with increasing IAP and, after 10 h or more IAP, usually resulted in 100% transmission (Table 2). Table 2 shows that *A. cajani* required a minimum of 90 min IAP to transmit PPSMV, and close to 100% transmission occurs when the IAP is over 5 h.

AAP. Single nonviruliferous *A. cajani* acquired PPSMV after a minimum of 15 min AAP and transmitted to 13% of the plants, but increasing the AAP increased transmission to a maximum of 53% (Table 3). Using groups of 10 mites, the minimum AAP for PPSMV transmission remained at 15 min with 64% of plants becoming infected. Increasing the AAP resulted in a mean of 76% transmission of PPSMV after 30 min AAP and >84% at 2 h and above (Table 3). None of the plants inoculated with *A. cajani* exposed for 5 and 10 min AAP were infected, indicating that a minimum of 15 min AAP is required for *A. cajani* to acquire PPSMV.

Latent period. When single *A. cajani* were transferred to healthy pigeon pea seedlings immediately after a 45-min AAP, none of the plants exposed for 1 h or less (IAP) were infected, confirming that *A. cajani* requires a minimum 90 min IAP (Table 2). There was no difference in this minimum IAP required to transmit PPSMV to pigeon pea between mites that were given short (minutes) or long (hours) AAP (Table 3), indicating that there is no detectable latent period associated with virus transmission.

Retention period. Single viruliferous *A. cajani* transmitted PPSMV to plants for up to two serial transfers when the IAP was from 2 to 6 h between each transfer (Table 4). PPSMV was transmitted only in the first transfer when the IAP was 10 or more hours between each transfer, indicating that *A. cajani* loses virus when fed for >10 h on a healthy plant. None of the plants exposed to viruliferous *A. cajani* for 1 h were infected in serial transfers, confirming that continuous feeding on a plant for 90 min is essential for successful virus transmission (Tables 2 and 4).

Effect of pre- and post-acquisition starvation of *A. cajani* on PPSMV transmission. *A. cajani* starved on dried pigeon pea leaflets survived and were active without feeding up to a maximum period of 13 h at room temperature (Table 5). However, after this period, movement of starved mites was sluggish, they turned a reddish-brown color, and their size was reduced considerably. Transmission experiments using starved mites indicated that starvation decreased both AAP and IAP. Nonviruliferous *A. cajani* starved for 3 to 4 h prior to virus acquisition acquired PPSMV in a minimum AAP of 10 min (Table 6). Similarly, viruliferous mites starved for 3 to 4 h before exposing them to healthy pigeon pea transmitted PPSMV in an IAP of 1 h (Table 7). Viruliferous mites starved for up to 13 h retained the ability to transmit (Table 5).

Survival of *A. cajani* on healthy and PPSMV-infected pigeon pea. When mite numbers were counted on PPSMV-infected and healthy pigeon pea plants 2 months after infesting them with nonviruliferous *A. cajani,* there were far fewer mites (7 to 8 mites per leaflet) on healthy than on PPSMV-infected plants (>50 mites per leaflet).

Table 5. Effect of starvation of *Aceria cajani* on transmission of Pigeon pea sterility mosaic virus (PPSMV) for groups of 10 mites given an acquisition access period of 24 h and then starved for different times[a]

	No. plants infected/no. plants tested (% transmission)		
Starvation period (h)	Replicate 1	Replicate 2	Mean %
1	3/12 (25)	4/16 (25)	25
3	4/15 (26)	3/16 (18)	23
4	2/13 (15)	2/15 (13)	14
6	2/11 (18)	2/14 (14)	16
9	2/9 (22)	3/11 (27)	25
13	2/11 (18)	2/7 (28)	22

[a] Mite feeding was not terminated after transfer to test plants for inoculation access period. Test plants were assayed for PPSMV by double-antibody sandwich enzyme-linked immunosorbent assay 3 weeks postinoculation.

Table 6. Effect of starvation of *Aceria cajani* on transmission of Pigeon pea sterility mosaic virus (PPSMV) for individual mites starved 3 to 4 h after acquisition access period and then transferred serially

	No. plants infected/no. plants tested (% transmission)		
Serial IAP[a]	Transfer 1	Transfer 2	Transfer 3
30 min	0/9 (0)	0/7 (0)	0/7 (0)
1 h	5/9 (55)	3/8 (37)	1/7 (14)
2 h	3/12 (25)	1/11 (9)	2/9 (22)

[a] Inoculation access period.

Table 7. Effect of starvation of *Aceria cajani* on transmission of Pigeon pea sterility mosaic virus (PPSMV) for individual nonviruliferous mites starved 3 to 4 h prior to acquisition access period (AAP)

	No. plants infected/no. plants tested (% transmission)			
AAP	Replicate 1	Replicate 2	Replicate 3	Mean %
5 min	0/10 (0)	0/12 (0)	0/10 (0)	0
10 min	2/12 (16)	3/11 (36)	1/8 (12)	19
15 min	3/11 (36)	1/10 (10)	2/11 (18)	19
30 min	2/13 (15)	3/10 (30)	2/10 (20)	21
1 h	3/9 (33)	2/8 (25)	3/9 (33)	31
3 h	2/8 (25)	3/10 (30)	3/10 (30)	29
5 h	3/11 (27)	3/10 (30)	2/8 (25)	28

DISCUSSION

In this study, it was essential to produce and maintain a PPSMV-free culture of *A. cajani.* The use of floating leaflets of a PPSMV-immune cultivar proved to be a simple and efficient means to generate nonviruliferous mites within 3 days. Previous methods that used plants of a PPSMV-resistant pigeon pea cultivar took more than 3 months to generate nonviruliferous *A. cajani,* and the populations obtained after that period remained low (4).

Our study has shown that single *A. cajani* transmitted PPSMV, but that transmission efficiency was not 100% unless 10 mites per plant were used (Tables 1 to 3). Increased AAPs increased transmission of PPSMV by single *A. cajani*, but the maximum transmission achieved with single mites was about 50% (Table 3). However, this compares with the much lower efficiency reported for the transmission of some other mite-transmitted viruses. For example, only 17% of individual *Eriophyes insidiosus* transmitted *Peach mosaic virus* (PMV) (5), and 3 to 11% of adult and c. 50% of second instars of *A. tosichella* transmitted *Wheat streak mosaic virus* (WSMV) (18). Compared with these other virus-vector mite species, therefore, *A. cajani* is a relatively efficient vector of PPSMV. One possible reason for the low efficiency of transmission by single mites may be differing efficiencies in the acquisition/transmission of mites at different stages in their life cycle (18). Another is that different populations of individual mite species may differ in vector efficiency, as has been shown recently for the transmission of HPV by *A. tosichella* (26).

For successful transmission of PPSMV, *A. cajani* required a minimum of 15 min AAP and 90 min IAP, but these times were decreased to 10 min and 60 min, respectively, when mites were starved prior to feeding (Tables 2, 3, and 5 to 7). These differences in time following starvation were probably due to the immediate feed-

ing of starved mites soon after they were transferred onto fresh leaves. Under normal transfer conditions, mites spent some time searching for a suitable site on the leaf surface before feeding. Viruliferous mites lost the ability to transmit PPSMV after feeding for 2 to 10 h on healthy plants (Table 4), and there was no apparent latent period associated with transmission. It is possible that mites inoculate PPSMV immediately on feeding but that a minimum of 90 min feeding is required to inject sufficient virus for infection to occur.

A. cajani retained PPSMV for up to 6 h when feeding and for more than 13 h without access to a susceptible host (Tables 4 to 7). This explains the ability of *A. cajani* to transmit PPSMV after being carried in wind currents to new plants. Although *A. cajani* remained alive without feeding for up to 30 h in a moist chamber, they did not survive when transferred to plants. Therefore, it is unlikely that in nature the mites survive for very many hours without feeding. Viruliferous *A. cajani* did not retain PPSMV for life as shown by serial transmission studies using single mites (Table 4), confirming an earlier study (24). It is not surprising therefore that we found no evidence for transovarial transmission. Indeed, none of the eriophyid mite-borne viruses are reported to multiply in their vector, nor are they transmitted through the egg of their vector (16).

Transmission details for most other eriophyid mite-borne viruses are not well defined due to the inherent difficulties in manipulating such tiny creatures. To date, the best-studied relationship is that of WSMV and its vector *A. tosichella* (previously known as *A. tulipae*). WSMV is transmitted by all stages of its vector, and is retained through the molt, but not through the egg. However, adults transmit only if they acquire virus during their immature stages; they cannot acquire the virus as adults and then transmit it. *A. tosichella* acquires WSMV in a minimum AAP of 15 min (29), although 50% transmission of WSMV was only achieved with AAP of 16 h (18). Once acquired, WSMV is transmitted by *A. tosichella* for at least 4 days after transferring them from WSMV-immune plants back to WSMV-susceptible plants (3). Furthermore, ultrastructural studies showed WSMV particles in the midgut of viruliferous mites, where they persisted for at least 5 days (20,29); occasionally particles were also found in the hemocoel and salivary glands (19). Based on these findings, it was suggested that the mode of transmission was circulative, but transmission by regurgitation, although unlikely, was not ruled out (19). In less detailed studies, the uncharacterized agent of fig mosaic disease was reported to be transmitted in a persistent manner by *A. ficus* with a 6- to 7-h latent period in the vector, to be retained through the molt, and to be transmitted by viruliferous mites for up to 10 days (21,22). However, no latent period is reported for any other eriophyid mite-borne plant pathogen (16).

Based on our data, the transmission of PPSMV by *A. cajani* is best considered to be in a semipersistent manner. Studies on two other mite-transmitted viruses also indicate a semipersistent mode of transmission. Thus, transmission of PMV by *E. insidiosus* requires an AAP of 3 days and an IAP of 6 h with no latent period (5), and of *Ryegrass mosaic virus* by *Abacarus hystrix* requires an AAP of at least 2 h and mites lose infectivity after 24 h (13,30).

PPSMV has several novel properties (7,9), such as in particle morphology and composition and ultrastructural effects in plants, and shows close similarities with HPV and to disease agents transmitted by eriophyid mites that cause rose rosette and fig mosaic (2,7,9,16). In the light of our findings, a reassessment of the suggested persistent transmission of the agent of fig mosaic disease by *A. ficus* may be worthwhile.

Finally, the finding that PPSMV infection of pigeon pea greatly increased the reproduction of *A. cajani* compared with healthy plants confirms earlier field observations (25). Similarly, greatly increased numbers of *Cecidophyopsis ribis*, the mite vector of *Blackcurrant reversion virus*, are reported on blackcurrant plants infected with this virus compared with healthy plants (31). In these instances, therefore, there is a beneficial relationship between the vector mite and the virus it transmits.

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LITERATURE CITED

1. Ahn, K.-K., Kim, K. S., Gergerich, R. C., and Jensen, S. G. 1998. High plains virus of corn and wheat: Ultrastructural and serological aspects. J. Submicrosc. Cytol. Pathol. 30:563-571.
2. Ahn, K.-K., Kim, K. S., Gergerich, R. C., Jensen, S. G., and Anderson, E. J. 1996. Comparative ultrastructure of double membrane-bound particles and inclusions associated with eriophyid mite-borne plant diseases of unknown etiology: A potentially new group of plant viruses. J. Submicrosc. Cytol. Pathol. 28:345-355.
3. Del Rosario, M. S., and Sill, W. H., Jr. 1965. Physiological strains of *Aceria tulipae* and their relationships to the transmission of wheat streak mosaic virus. Phytopathology 55:1168-1175.
4. Ghanekar, A. M., Sheila, V. K., Beniwal, S. P. S., Reddy, M. V., and Nene, Y. L. 1992. Sterility mosaic of pigeonpea. Pages 415-428 in: Plant Diseases of International Importance: Diseases of Cereals and Pulses, Vol. I. U. S. Singh, A. N. Mukhooadhyay, J. Kumar, and H. S. Chaubey, eds. Prentice Hall, Upper Saddle River, NJ.
5. Gispert, C., Oldfield, G. N., Perring, M. T., and Creamer, R. 1998. Biology of the transmission of peach mosaic virus by *Eriophyes insidiosus* (Acari: Eriophyidae). Plant Dis. 82:1371-1374.
6. Kannaiyan, J., Nene, Y. L., Reddy, M. V., Ryan, J. G., and Raju, T. N. 1984. Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa, and America. Trop. Pest Manag. 30:62-71.
7. Kumar, P. L., Duncan, G. H., Roberts, I. M., Jones, A. T., and Reddy, D. V. R. 2002. Cytopathology of Pigeonpea sterility mosaic virus in pigeonpea and *Nicotiana benthamiana*: Similarities with those of eriophyid mite-borne agents of undefined aetiology. Ann. Appl. Biol. 140:87-96.
8. Kumar, P. L., Fenton, B., Duncan, G., Jones, A. T., Sreenivasulu, P., and Reddy, D. V. R. 2001. Assessment of variation in *Aceria cajani* (Acari: Eriophyidae) using analysis of nuclear rDNA ITS regions and scanning electron microscopy: Implications for the variability observed in host plant resistance to pigeonpea sterility mosaic disease. Ann. Appl. Biol. 139:61-73.
9. Kumar, P. L., Jones, A. T., Duncan, G. H., Roberts, I. M., and Reddy, D. V. R. 2001. Characterisation of a novel mite-transmitted virus associated with pigeonpea sterility mosaic disease. (Abstr.) Phytopathology 91:S51.
10. Kumar, P. L., Jones, A. T., and Reddy, D. V. R. 2002. Mechanical transmission of Pigeonpea sterility mosaic virus. Plant. Pathol. 32:88-89.
11. Kumar, P. L., Jones, A. T., and Reddy, D. V. R. 2002. Methods for the detection of Pigeonpea sterility mosaic virus and screening for SMD resistance – Training course manual. ICRISAT Publication, Patancheru, India.
12. Mirabile, J., Scholthof, K.-B. G., and Scholthof, H. B. 2001. Biological studies and molecular characterization of the High Plains disease pathogen. (Abstr.) Phytopathology 91:S63.
13. Mulligan, T. E. 1960. The transmission by mites, host range and properties of ryegrass mosaic virus. Ann. Appl. Biol. 48:575-579.
14. Muniyappa, V., and Nangia, N. 1982. Pigeonpea cultivars and selections for resistance to sterility mosaic in relation to prevalence of eriophyid mite *Aceria cajani* Channabasavanna. Trop. Grain Legume Bull. 25:28-30.
15. Nene, Y. L., and Reddy, M. V. 1976. Screening for resistance to sterility mosaic of pigeonpea. Plant Dis. Rep. 66:1034-1036.
16. Oldfield, G. N., and Proeseler, G. 1996. Eriophiod mites as vectors of plant pathogens. Pages 259-275 in: Eriophyoid Mites - Their Biology, Natural Enemies and Control. E. E. Lindquist, M. W. Sabelis, and J. Bruin, eds. Elsevier Science B. V., The Netherlands.
17. Oldfield, G. N., Reddy, M. V., Nene, Y. L., and Reed, W. 1981. Preliminary studies of the eriophyid vector of sterility mosaic. Int. Chickpea Pigeonpea Newsl. 1:25-27.
18. Orlob, G. B. 1966. Feeding and transmission characteristics of *Aceria tulipae* Kiefer as vector of wheat streak mosaic virus. Phytopathol. Z. 55:218-238.
19. Paliwal, Y. C. 1980. Relationship of wheat streak mosaic and barley stripe mosaic viruses to vector and non-vector eriophyid mites. Arch. Virol. 63:123-132.
20. Paliwal, Y. C., and Slykhuis, J. T. 1967. Localization of wheat streak mosaic virus in the alimentary canal tract of its vector, *Aceria tulipae* K. Virology 32:344-353.
21. Proeseler, G. 1969. Transmission of fig mosaic virus by the gall mite *Aceria ficus* (Cotte). Zbl. Bakteriol. Parasitenkede. Intekt. Krank. Hyg. Abt.II. Naturw. 123:288-292.
22. Proeseler, G. 1972. Relationships between virus, vector and host plant shown by fig mo-

saic virus and *Aceria ficus* (Cotte). Acta Phytopathol. Acad. Sci. Hung. 7:179-186.
23. Reddy, A. S., Kulkarni, N. K., Kumar, P. L., Jones, A. T., Muniyappa, V., and Reddy, D. V. R. 2002. Improved graft inoculation method for screening resistance to Pigeonpea sterility mosaic virus. Int. Chickpea Pigeonpea Newsl. In press.
24. Reddy, M. V., Beniwal, S. P. S., Sheila, V. K., Sithananthan, S., and Nene, Y. L. 1989. Role of eriophyid mite (A*ceria cajani*) in transmission and spread of sterility mosaic of pigeonpea. Pages 121-127 in: Progress in Acarology. G. P. Channbasavanna and C. A. Virakthmath, eds. Oxford and IBH Publishing Co., New Delhi, India.
25. Reddy, M. V., and Nene, Y. L. 1980. Influence of sterility mosaic resistant pigeonpeas on multiplication of the mite vector. Ind. Phytopathol. 33:61-63.
26. Seifers, D. L., Harvey, T. L., Louie, R., Gordon, D. T., and Martin, T. J. 2002. Differential transmission of isolates of the *High Plains virus* by different sources of wheat curl mites. Plant Dis. 86:138-142.
27. Seth, M. L. 1962. Transmission of pigeonpea sterility by an eriophyid mite. Ind. Phytopathol. 15:225-227.
28. Singh, A. K., Rathi, Y. P. S., and Agrawal, K. C. 1999. Sterility mosaic of pigeonpea: A challenge of 20th Century. Ind. J. Virol. 15:85-92.
29. Slykhuis, J. T. 1955. *Aceria tulipae* in relation to the spread of wheat streak mosaic. Phytopathology 45:116-128.
30. Slykhuis, J. T., and Paliwal, Y. C. 1972. Ryegrass mosaic virus. CMI/AAB Descr. Plant Viruses No. 86.
31. Thresh, J. M. 1964. Increased susceptibility to the mite vector (*Phytoptus ribis* Nal.) caused by infection with black-currant reversion virus. Nature, Lond. 202:1028.