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Drake C. Stenger
University of Nebraska - Lincoln

Gary L. Hein
University of Nebraska Panhandle Research Center, Scottsbluff, Nebraska, ghein1@unl.edu

Frederick E. Gildow
Pennsylvania State University, University Park, Pennsylvania

Kempton M. Horken
University of Nebraska - Lincoln, khorken2@unl.edu

Roy C. French
University of Nebraska - Lincoln, rfrench2@unl.edu

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Plant Virus HC-Pro Is a Determinant of Eriophyid Mite Transmission

Drake C. Stenger,[1]* Gary L. Hein,[2] Frederick E. Gildow,[3] Kempton M. Horken,[1] and Roy French[1]

United States Department of Agriculture—Agricultural Research Service, and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583[1]; Department of Entomology, University of Nebraska Panhandle Research Center, Scottsbluff, Nebraska 69361[2]; and Department of Plant Pathology, Buckhout Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802[3]

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The eriophyid mite transmitted *Wheat streak mosaic virus* (WSMV; genus *Tritimovirus*, family *Potyviridae*) shares a common genome organization with aphid transmitted species of the genus *Potyvirus*. Although both tritimoviruses and potyviruses encode helper component-proteinase (HC-Pro) homologues (required for nonpersistent aphid transmission of potyviruses), sequence conservation is low (amino acid identity, ~16%), and a role for HC-Pro in semipersistent transmission of WSMV by the wheat curl mite (*Aceria tosichella* [Keifer]) has not been investigated. Wheat curl mite transmissibility was abolished by replacement of WSMV HC-Pro with homologues of an aphid transmitted potyvirus (*Turnip mosaic virus*), a rymovirus (*Agropyron mosaic virus*) vectored by a different eriophyid mite, or a closely related tritimovirus (*Oat necrotic mottle virus*; ONMV) with no known vector. In contrast, both WSMV-Sidney 81 and a chimeric WSMV genome bearing HC-Pro of a divergent strain (WSMV-El Batán 3; 86% amino acid sequence identity) were efficiently transmitted by *A. tosichella*. Replacing portions of WSMV-Sidney 81 HC-Pro with the corresponding regions from ONMV showed that determinants of wheat curl mite transmission map to the 5′-proximal half of HC-Pro. WSMV genomes bearing HC-Pro of heterologous species retained the ability to form virions, indicating that loss of vector transmissibility was not a result of failure to encapsidate. Although titer in systemically infected leaves was reduced for all chimeric genomes relative to WSMV-Sidney 81, titer was not correlated with loss of vector transmissibility. Collectively, these results demonstrate for the first time that HC-Pro is required for virus transmission by a vector other than aphids.

Most plant viruses utilize biological vectors to mediate horizontal transmission from host to host. Although plant virus coat proteins are determinants of vector transmission, aphid transmitted species of the genus *Potyvirus* also require the helper component-proteinase (HC-Pro) (for reviews see references 20 and 36). Potyvirus HC-Pro mediates aphid transmission through protein-protein interactions, serving as a bridge between the coat protein of virions and surfaces of the aphid maxillary food canal and foregut (1, 3, 5, 6, 24, 39).

The plant virus family *Potyviridae* (4) includes genera with members transmitted by aphids (*Potyvirus* and *Macluravirus*), whiteflies (*Ipomovirus*), fungal-like protists (*Bymovirus*), and eriophyid mites (*Rymovirus* and *Tritimovirus*). Rymoviruses and tritimoviruses encode HC-Pro homologues that lack motifs known to be required for vector transmission of potyviruses (5, 24). Complete nucleotide sequences for two ipomoviruses have been determined: *Sweet potato mild mottle virus* encodes an HC-Pro homologue (14), but surprisingly *Cucumber vein yellowing virus* does not (25). No obvious HC-Pro homologue is encoded by the bipartite bymoviruses and, as there are no complete nucleotide sequences available, it is unknown if macluraviruses encode an HC-Pro homologue. Presently, data concerning viral determinants of vector transmission are available only for aphid transmitted species of the genus *Potyvirus* and it is not known if HC-Pro homologues encoded by non-aphid-transmitted viruses of the family *Potyviridae* also mediate transmission by their respective vectors.

The potyvirus HC-Pro is a multifunctional protein not only required for aphid transmission but also for viral polyprotein processing (7, 8, 28, 37), long-distance systemic movement within infected plants (15, 30, 42), maintenance of viral RNA genome amplification (15, 30, 31), synergistic interactions with unrelated viruses in mixed infections (40), and suppression of posttranscriptional gene silencing (2, 9, 29, 32, 50). While it appears that the cysteine proteinase domain located in the carboxy-terminal region of HC-Pro is conserved among genera, such that polyprotein processing likely occurs similarly, low sequence conservation in the amino-proximal two-thirds of the protein results in an ambiguous alignment (18) with correspondingly low confidence in prediction of function. Recently, it was demonstrated (47) that the tritimovirus *Wheat streak mosaic virus* (WSMV) tolerated replacement of the HC-Pro cistron with the corresponding cistron from evolutionarily diverse species within the family *Potyviridae*. These observations suggest that despite limited sequence identity, any function(s) of HC-Pro necessary for infection of wheat by WSMV is provided by HC-Pro homologues.

WSMV is transmitted by the wheat curl mite (*Aceria tosichella* [Keifer]) (44). Unlike nonpersistent transmission of potyviruses by aphids, vector transmission of WSMV is semipersistent. Wheat curl mite juveniles may acquire the virus and remain viruliferous for days following acquisition, even after molting to the adult stage (45). Because chimeric WSMV genomes bearing HC-Pro from a variety of sources retained the

* Corresponding author. Mailing address: United States Department of Agriculture—Agricultural Research Service and Department of Plant Pathology, 344 Keim Hall, University of Nebraska, Lincoln, NE 68583. Phone: (402) 472-2710. Fax: (402) 472-4020. E-mail: dstenger@unlnotes.unl.edu.

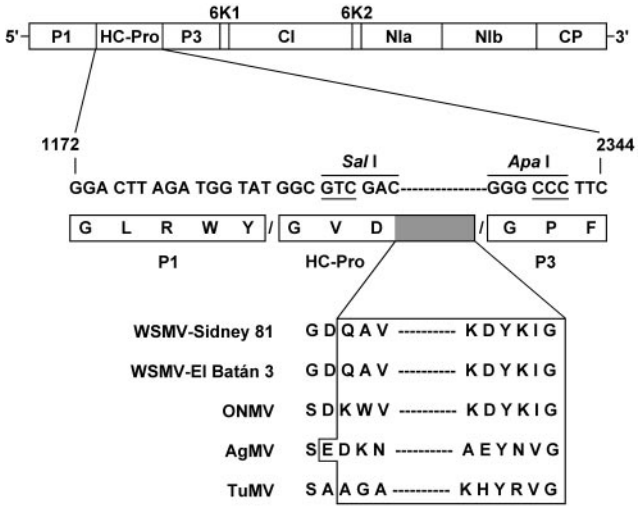

FIG. 1. Schematic representation of HC-Pro cistron replacements in the *Wheat streak mosaic virus*-Sidney 81 (WSMV-S81) genome. Locations of two 3-base insertions (underlined) introduced into pS81-SA to generate SalI and ApaI sites bordering the HC-Pro cistron are indicated. Sequences corresponding to the upstream and downstream junctions of the HC-Pro cistron are indicated as codon triplets (presented as cloned DNA) with the corresponding amino acid residues denoted by one letter code. Slashes (/) denote proteinase cleavage sites, a gray bar denotes HC-Pro sequences replaced with the corresponding sequences of another strain of WSMV (El Batán 3) or the distinct species *Turnip mosaic virus* (TuMV; genus *Potyvirus*), *Agropyron mosaic virus* (AgMV; genus *Rymovirus*), or *Oat necrotic mottle virus* (ONMV; genus *Tritimovirus*).

ability to systemically infect wheat following mechanical inoculation (47), we used this system to evaluate the role of HC-Pro in transmission of WSMV by the wheat curl mite. In this report we describe vector transmission experiments using chimeric WSMV genomes bearing HC-Pro replacements derived from an aphid transmitted potyvirus (*Turnip mosaic virus* [TuMV] [26]), a rymovirus vectored by a different eriophyid mite (*Agropyron mosaic virus* [AgMV] [18]), a more closely related tritimovirus with no known vector (*Oat necrotic mottle virus* [ONMV] [46]), and a divergent strain of WSMV (WSMV-El Batán 3) known to be transmitted by the wheat curl mite (11, 43). We further mapped the domain of HC-Pro required for wheat curl mite transmission by using WSMV constructs bearing portions of HC-Pro derived from ONMV.

MATERIALS AND METHODS

Construction of chimeric WSMV genomes. A full-length cDNA clone of the WSMV-Sidney 81 (WSMV-S81) genome from which infectious in vitro transcripts may be derived has been described previously (10). The WSMV-S81 full-length clone has been modified (pS81-SA) to accept HC-Pro replacement cassettes by the addition of SalI and ApaI sites (Fig. 1) without altering infectivity, pathogenicity, or host range (47). Full-length cDNA clones of chimeric WSMV genomes with the HC-Pro cistron of pS81-SA replaced with the corresponding cistron derived from WSMV-El Batán 3 (pS81EBHC-2), ONMV (pS81ONHC21-2), AgMV (pS81AGHC18-1-6), or TuMV (pS81TMHC10-1) have been constructed (Fig. 1) and demonstrated to serve as templates for generation of transcripts infectious to wheat (47). For both S81-SA and all chimeric WSMV genomes, the amino terminus of HC-Pro is cleaved from the polyprotein by the WSMV-S81 P1 serine proteinase (12) and (except for the AgMV HC-Pro replacement) bears the first two amino-terminal residues of WSMV-S81 (glycine and aspartate) separated by an introduced valine residue encoded by three nucleotides inserted to generate the SalI site in pS81-SA. Since the 5′-proximal end of the AgMV HC-Pro cistron replacement cassette was inserted into pS81-SA as an XhoI end ligated to the SalI end of pS81-SA (47), the amino terminus of the AgMV HC-Pro protein replacement also contained the WSMV-S81 terminal glycine and introduced valine residues followed by the second amino acid residue of AgMV (glutamate) instead of WSMV-S81 (aspartate). All HC-Pro amino acid residues downstream of position 2 through to the carboxy terminus were derived from the heterologous virus such that the active site of the HC-Pro cysteine proteinase was paired with the cognate proteinase cleavage site at the carboxy terminus. Downstream of HC-Pro, all sequences corresponded to WSMV-S81 and included a proline residue (encoded by three nucleotides inserted to generate the ApaI site) located between the first and second amino-terminal residues of P3 (Fig. 1).

As WSMV-S81 and ONMV share considerable sequence identity (72.9% nucleotide; 80% amino acid) and the HC-Pro cistrons of the two viruses may be aligned unambiguously and without gaps (46), chimeric HC-Pro cistrons could be constructed by altering the sequence of one virus to contain the same endonuclease restriction site naturally present in the other virus. Two reciprocal pairs of clones, bearing HC-Pro sequences derived from both WSMV-S81 and ONMV fused at either the Csp45I site (nucleotide [nt] 1438) of ONMV or the NheI site (nt 1816) of WSMV-S81 were generated by substituting wild-type fragments of one virus genome with PCR generated fragments (having the appropriate restriction endonuclease site for fusion engineered into the primer sequence) of the other virus genome. The resulting chimeric HC-Pro cassettes were verified by sequencing and subsequently used to replace the wild-type SalI-ApaI fragment of pS81-SA to produce pS81SCOHC2-2, pS81SNOHC1-3, pS81OCSHC4-1, and pS81ONSHC1-4 (Fig. 2A).

Transcripts of pS81-SA and each chimeric viral construct were prepared and mechanically inoculated to wheat seedlings as described previously (10), except that carborundum was used as an abrasive. Verification that transcript-inoculated plants were infected with the intended genotype was accomplished by restriction endonuclease digestion of reverse transcription-PCR (RT-PCR) products of the HC-Pro cistron (47) amplified from total nucleic acid samples extracted from plants as described (34). RT-PCR was conducted using the primer HCR (Sidney 81 nt 2373 to 2345) for RT and the primer pair HCF (Sidney 81 nt 1159 to 1186) and HCR for PCR as described (47).

Eriophyid mite transmission assays. Source plants for vector transmission experiments were prepared by mechanical inoculation of wheat seedlings (10 to 12 days old) using sap extracted from systemically infected, transcript-inoculated plants. Approximately 2 weeks postinoculation, groups of wheat curl mites (~10/plant) from an aviruliferous colony were transferred to individually caged source plants. Wheat curl mite populations increased on source plants during a 3-week acquisition access period before being transferred in groups of 10 to individually caged test plants (10- to 12-day-old seedlings, five test plants/source plant). Source plants mechanically inoculated and infected with progeny virus derived from pS81-SA were used as positive controls for vector transmission assays. Source plants not mechanically inoculated with virus served as negative controls for vector transmission assays. Wheat curl mite populations were allowed a 3- to 4-week inoculation access period (IAP) on test plants. Following the IAP, wheat curl mite population density on test plants was estimated visually, and leaf tissue samples were collected and stored at −80°C until assayed. Infection status of both source plants and test plants was assessed by RT-PCR of the HC-Pro cistron as described above, followed by digestion with restriction endonucleases diagnostic for each HC-Pro cistron (47) (Fig. 2).

Electron microscopy. Virions were concentrated and partially purified from 5-g leaf samples. Infected leaf tissue was ground in a mortar and pestle with 20 ml of 0.1 M potassium phosphate buffer (pH 7.0) and sterile sand. Chloroform (10 ml) was added and the homogenate was vortexed for 10 min. Following centrifugation (10 min, 4°C, 4,300 × *g*), the aqueous phase was recovered, adjusted to 8% polyethylene glycol (PEG) and 0.2 M NaCl, and incubated at 0°C for 1 h. PEG precipitants were concentrated by centrifugation (10 min, 4°C, 7,700 × *g*) and resuspended in 1 ml 0.01 M phosphate buffer. Following centrifugation (10 min, 4°C, 9,300 × *g*), the supernatant containing partially purified virions in suspension was retained for serologically specific electron microscopic (SSEM) analysis (23, 35).

Copper grids (300 mesh) were coated with formvar and carbon and then incubated on WSMV antiserum diluted 1:400 for 1 h at room temperature. Coated grids were rinsed with phosphate-buffered saline–Tween 20 and then floated on drops (20 μl) of partially purified virion suspensions for 1.5 h at 4°C and rinsed again in PBS-Tween. Virions adhering to antibody coated grids were fixed with 2% glutaraldehyde for 10 min, rinsed twice with distilled water, and negatively stained in 2% uranyl acetate for 3 min. Excess uranyl acetate was removed, and the grids were air-dried overnight and then examined for virions with a JEM 1200 EXII electron microscope (JEOL USA, Inc., Peabody, MA).

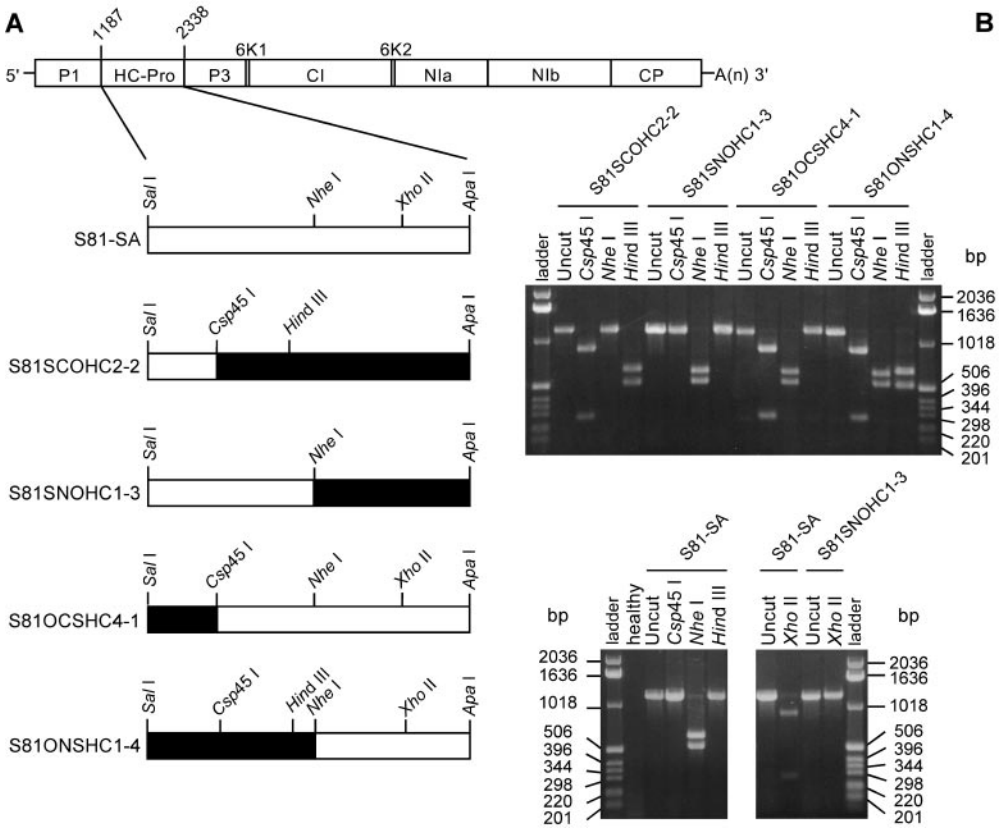

FIG. 2. *Wheat streak mosaic virus*-Sidney 81 (WSMV-S81) genomes with chimeric HC-Pro cistrons bearing domains from both WSMV-S81 (white regions) and *Oat necrotic mottle virus* (ONMV; black regions). Panel A is a schematic representation of the WSMV-S81 genomes bearing chimeric WSMV-S81/ONMV HC-Pro cistrons fused at either the Csp45I or NheI sites. Panel B depicts restriction endonuclease profiles of RT-PCR products of the HC-Pro cistron amplified from total RNA samples extracted from plants infected with each viral genome displayed in Panel A.

Estimation of relative virus titer. Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was conducted with a commercially available kit (Agdia, Elkhart, IN) following the manufacturer's recommendations. Systemically infected, symptomatic wheat leaves (16 days post inoculation) collected from five plants were pooled and stored at −80°C until processed. Similar leaf samples were collected from uninoculated plants as negative controls. For each pooled sample, two subsamples (0.5 g) were ground in 5 ml of ELISA extraction buffer. Duplicate series of twofold serial dilutions of plant sap (50, 25, 12.5, 6.25, and 3.125 mg plant tissue/ml) for each subsample were applied to wells of microtiter plates previously coated with antibodies raised against WSMV virions. Mean ELISA absorbance values (405 nm) were calculated using four measurements (two replications of two subsamples) for each dilution of plant sap. The mean absorbance value calculated for each dilution of uninfected sap was subtracted from the corresponding mean absorbance value for each dilution of virus-infected sap. These corrected absorbance values were plotted as a function of log plant sap concentration for S81-SA and each chimeric virus. Using the absorbance versus log plant sap concentration plot of S81-SA as a standard, the titer of each chimeric virus relative to S81-SA was determined for each plant sap dilution for which mean absorbance values exceeded that of the most dilute plant sap from S81-SA infected leaves. Relative titer of each chimeric virus was expressed as a percentage of S81-SA and was based on four estimates for each chimeric virus except S81SCOHC2-2, for which only the two highest plant sap concentrations yielded mean absorbance values in excess of that measured for the most dilute plant sap from S81-SA infected leaves.

RESULTS

HC-Pro replacements from other virus species abolish wheat curl mite transmission. Eriophyid mite transmission assays conducted with S81-SA or WSMV-S81 bearing HC-Pro replacements are summarized in Table 1. Vector transmission of S81-SA ranged from 100% (5 out of 5 test plants infected) to 60% (3 out of 5 test plants infected) from individual source plants, with an overall transmission efficiency of 90% (36 out of 40 test plants infected). The chimeric WSMV-S81 genome bearing HC-Pro from WSMV-El Batán 3 was transmitted from each source plant to as many as 5 out of 5 (100%) test plants or to as few as 2 out of 5 (40%) test plants, with an overall transmission efficiency of 85% (34 out of 40 test plants infected). Verification that infected test plants harbored the same genotype (e.g., HC-Pro of S81 or El Batán 3) as the source plants from which wheat curl mites acquired virus was accomplished by a diagnostic SspI digestion (47) of RT-PCR products amplified from individual test plants (data not shown). No vector transmission was observed for chimeric WSMV genomes with HC-Pro derived from TuMV (0 out of 40 test plants infected), AgMV (0 out of 40 test plants infected), or ONMV (0 out of 35 test plants infected), with all test plants remaining asymptomatic and all test plants assayed as negative for viral infection by RT-PCR (data not shown). No infection of test plants (0 out of 40) colonized by wheat curl mites transferred from uninfected source plants was detected by visual observation for symptoms or by RT-PCR (data not shown).

TABLE 1. Wheat curl mite transmission assays of *Wheat streak mosaic virus*-Sidney 81 (WSMV-S81) genomes bearing HC-Pro cistron replacements

Source plant[b]	Transmission to test plants[a]					
	S81-SA[c]	S81EBHC-2[c]	S81ONHC21-2[c]	S81AGHC18-1-6[c]	S81TMHC10-1[c]	No virus
A	5/5	5/5	0/5	0/5	0/5	0/5
B	4/5	5/5	0/5	0/5	0/5	0/5
C	4/5	5/5	0/5	0/5	0/5	0/5
D	5/5	5/5	0/5	0/5	0/5	0/5
E	5/5	4/5	0/5	0/5	0/5	0/5
F	5/5	5/5	0/5	0/5	0/5	0/5
G	5/5	3/5	0/5	0/5	0/5	0/5
H	3/5	2/5	ND[d]	0/5	0/5	0/5

[a] Number of test plants infected/number of test plants inoculated by groups of 10 mites transferred from each source plant. All source and test plants were assayed for WSMV infection by RT-PCR.
[b] Source plants were mechanically inoculated with progeny virus derived from transcript-inoculated plants. Transmission assays for source plants A to D and E to H were conducted in separate experiments.
[c] Source of HC-Pro cistron was WSMV-Sidney 81 (S81-SA), WSMV-El Batán 3 (S81EBHC-2), *Oat necrotic mottle virus* (S81ONHC21-2), *Agropyron mosaic virus* (S81AGHC18-1-6), or *Turnip mosaic virus* (S81TMHC10-1).
[d] ND, not determined.

Essential domain(s) required for wheat curl mite transmission map to the 5′-proximal half of the HC-Pro cistron. In vitro transcripts of four WSMV genomes containing portions of the HC-Pro cistron derived from both WSMV-S81 and ONMV (Fig. 2A) were infectious when mechanically inoculated to wheat (Table 2) and produced systemic mosaic symptoms similar to those of S81-SA. The genotype of each WSMV-S81/ONMV HC-Pro chimera was verified by restriction endonuclease digestion of RT-PCR products from transcript inoculated plants (Fig. 2B). Wheat curl mite transmission (Table 3) and RT-PCR (data not shown) assays indicated that among the four WSMV-S81/ONMV HC-Pro chimeras, only S81SNOHC1-3 (containing the 5′-proximal half of HC-Pro from WSMV-S81 and the 3′-proximal half of HC-Pro from ONMV) was vector transmissible (28 out of 40 test plants infected based on RT-PCR assays). All test plants colonized by wheat curl mites given access to source plants infected with the three other WSMV-S81/ONMV HC-Pro chimeras assayed negative for viral infection by RT-PCR. Efficiency of vector transmission of S81SNOHC1-3 was more variable than that of S81-SA, ranging between 1 out of 5 (20%) to 5 out of 5 (100%) test plants infected per source plant. Overall vector transmission efficiency of S81SNOHC1-3 (70%) was moderately reduced relative to S81-SA (37 out of 40; 92.5%). Restriction endonuclease profiles of RT-PCR products verified that the S81SNOHC1-3 genotype was present in infected test plants and distinct from that of the positive control, S81-SA (data not shown). DNA sequencing of cloned RT-PCR products from three S81SNOHC1-3-infected test plants confirmed vector transmission of this chimeric WSMV genome (data not shown).

Mite populations readily increased on test plants. Because eriophyid mites are very small (0.2 by 0.04 mm) and delicate compared to aphids (Fig. 3), it is difficult to monitor feeding behavior and survival of individuals transferred from source plants to test plants. Therefore, to indirectly monitor the fate of wheat curl mites transferred from source plants, population density on test plants was estimated by visual inspection at time of leaf sample collection. Of 235 test plants colonized by 10 individuals/plant (Table 1), all but 5 test plants (98%) yielded wheat curl mite populations at least 10-fold greater than the

TABLE 2. Infectivity of *Wheat streak mosaic virus*-Sidney 81 (WSMV-S81) genomes with chimeric HC-Pro cistrons containing regions derived from both WSMV-S81 and *Oat necrotic mottle virus*

Transcription template	Infectivity[a]	
	Expt 1	Expt 2
pS81SCOHC2-2	9/10	7/10
pS81SNOHC1-3	10/10	10/10
pS81OCSHC4-1	10/10	10/10
pS81ONSHC1-4	10/10	10/10
pS81-SA[b]	9/10	10/10
None	0/10[c]	0/10[c]

[a] Number of wheat plants infected/number of wheat plants mechanically inoculated with RNA transcripts.
[b] HC-Pro cistron solely derived from WSMV-S81.
[c] Uninoculated plants.

TABLE 3. Wheat curl mite transmission assays of *Wheat streak mosaic virus*-Sidney 81 (WSMV-S81) genomes bearing chimeric HC-Pro cistrons containing portions derived from both WSMV-S81 and *Oat necrotic mottle virus*

Source plant[b]	Transmission to test plants[a]					
	S81-SA[c]	S81OCS HC4-1[c]	S81ONS HC1-4[c]	S81SNO HC1-3[c]	S81SCO HC2-2[c]	No virus
A	5/5	0/5	0/5	1/5	0/5	0/5
B	5/5	0/5	0/5	5/5	0/5	0/5
C	5/5	0/5	0/5	5/5	0/5	0/5
D	5/5	0/5	0/5	2/5	0/5	0/5
E	3/5	0/5	0/5	4/5	0/5	0/5
F	4/5	0/5	0/5	5/5	0/5	0/5
G	5/5	0/5	0/5	1/5	0/5	0/5
H	5/5	0/5	0/5	5/5	0/5	0/5

[a] Number of test plants infected/number of test plants inoculated by groups of 10 mites transferred from each source plant. All source and test plants were assayed for WSMV infection by RT-PCR.
[b] Source plants were mechanically inoculated with progeny virus derived from transcript- inoculated plants. Transmission assays for source plants A to D and E to H were conducted in separate experiments.
[c] Source of HC-Pro cistron was WSMV-Sidney 81 (S81-SA) or chimeric HC-Pro cistrons with regions derived from both WSMV-S81 and *Oat necrotic mottle virus*.

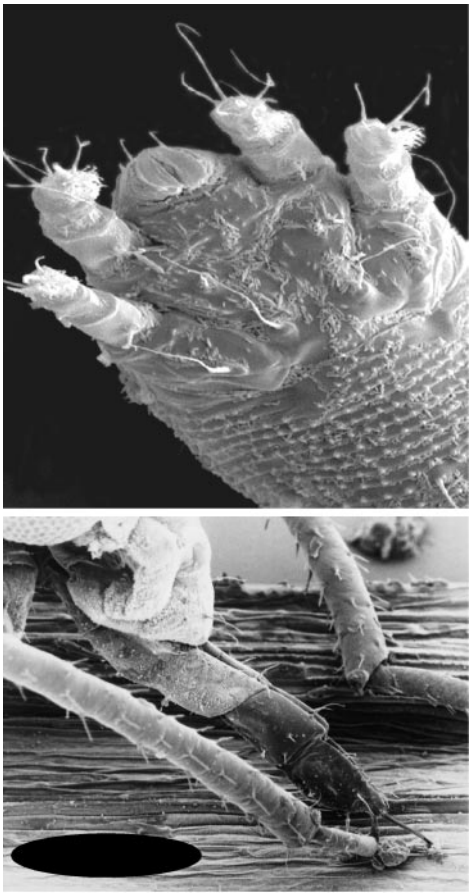

FIG. 3. Size comparison of plant virus arthropod vectors. Upper panel is a scanning electron micrograph of the anterior ventral surface of the wheat curl mite *Aceria tosichella* (Keifer) vector of *Wheat streak mosaic virus*. Lower panel is a scanning electron micrograph of the mouthparts and forelimbs of an aphid feeding on a leaf surface. Black oval (lower left) corresponds to size of a wheat curl mite (~0.2 mm by 0.04 mm) relative to the aphid depicted in the lower panel.

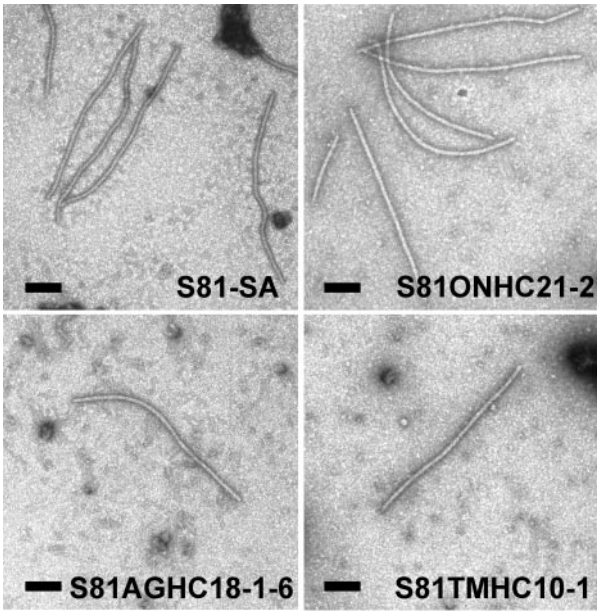

FIG. 4. Immunospecific electron microscopy of negatively stained virions extracted from plants infected with *Wheat streak mosaic virus*-Sidney 81 (S81-SA) or infected with WSMV-Sidney 81 bearing HC-Pro replacements derived from *Oat necrotic mottle virus* (S81ONHC21-2), *Agropyron mosaic virus* (S81AGHC18-1-6), or *Turnip mosaic virus* (S81TMHC10-1). Length of horizontal black scale bars corresponds to 50 nm.

cohort transferred from the source plant (data not shown). Two of the five test plants for which a 10-fold increase in wheat curl mite population density was not observed involved transfer of wheat curl mites from S81-SA-infected source plants. Both of these test plants became infected with S81-SA, indicating that viruliferous wheat curl mites transferred from source plants had fed on test plants. Similar results were obtained for vector transmission assays conducted with WSMV genomes bearing chimeric HC-Pro cistrons derived from both WSMV-S81 and ONMV. Visual estimates of wheat curl mite populations at the conclusion of the IAP for these experiments (Table 3) indicated that nearly all (99%) test plants supported greater than 10-fold increases in wheat curl mite populations (data not shown).

WSMV genomes bearing HC-Pro replacements are competent for virion assembly. It is presumed that virions are necessary for vector transmission of all plant viruses. Therefore, we examined extracts from wheat plants infected with S81-SA or WSMV bearing HC-Pro replacements from TuMV, AgMV, or ONMV. Although HC-Pro is not a structural component of the virion, it is possible that HC-Pro may be an accessory factor required for virion assembly, or that the RNA sequence may contain *cis*-acting elements necessary for encapsidation. SSEM revealed that typical flexuous, rod-shaped virions similar in length and appearance to wild-type WSMV were recovered from plants infected with chimeric viruses bearing HC-Pro replacements derived from TuMV, AgMV, or ONMV (Fig. 4). Virions of S81-SA and the chimeric WSMV genome with HC-Pro replaced by that of ONMV were abundant. In contrast, virions of the chimeric WSMV genomes with HC-Pro replacements from AgMV or TuMV were less abundant. No virions were observed by SSEM of extracts prepared from uninfected wheat plants (data not shown).

Titer of chimeric viruses is reduced relative to S81-SA but does not correlate with loss of vector transmissibility. ELISA was used to compare relative virus titer present in wheat leaves systemically infected with S81-SA or the various WSMV chimeric constructs evaluated for vector transmission. Titer of all chimeric viruses was reduced relative to S81-SA (Fig. 5). Viral genomes bearing HC-Pro of WSMV-El Batán 3 or ONMV had similar relative titers (not quite twofold less than S81-SA). Chimeric viruses bearing HC-Pro of TuMV or AgMV yielded titers approximately threefold less than S81-SA. Relative titer among the four viral genomes bearing chimeric HC-Pro derived from both Sidney 81 and ONMV varied from 16-fold less (S81SCOHC2-2) to not quite 2-fold less (S81OCSHC4-1) than S81-SA. Despite large differences in relative titer observed for the chimeric viruses, vector transmission competence was not correlated with virus concentration. In particular, relative titer of the vector transmissible genotype S81SNOHC1-3 (4.5-fold less than S81-SA) was substantially less than that of four non-vector-transmissible genotypes. Also, two non-vector-transmis-

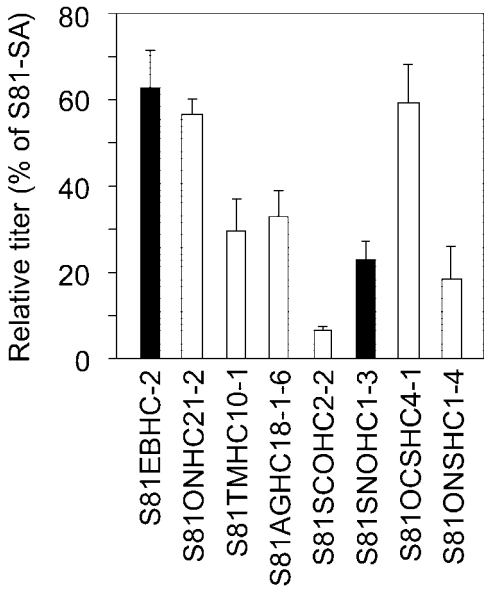

FIG. 5. Relative titer of chimeric viral genomes in systemically infected wheat leaves. Presented is a bar graph of relative virus titer (mean ± standard deviation expressed as a percentage of S81-SA) estimated for eight chimeric viral genomes. Black bars indicate viral genomes transmissible by the wheat curl mite. White bars indicate viral genomes not transmissible by the wheat curl mite vector.

sible genotypes (S81ONSHC21-2 and S81OCSHC4-1) yielded relative virus titers nearly equal to that of a vector-transmissible genotype (S81EBHC-2).

DISCUSSION

WSMV HC-Pro is a determinant of virus transmission by eriophyid mites. Efficiency of vector transmission of S81-SA or WSMV bearing the HC-Pro cistron of WSMV-El Batán 3 (Table 1) was similar to that of wild-type WSMV-S81 (10) or WSMV-El Batán 3 (43). As S81-SA was efficiently transmitted from source plants to test plants by wheat curl mites, the two codons inserted into pS81-SA to generate the SalI and ApaI sites necessary for HC-Pro cassette replacement (Fig. 1) did not interfere with vector transmission. Similarly, replacement of the WSMV-S81 HC-Pro cistron with that of WSMV-El Batán 3 had no significant effect on wheat curl mite transmission, indicating that HC-Pro of WSMV strains were functionally interchangeable.

Complete abolishment of vector transmission upon replacement of HC-Pro with the corresponding cistron of different virus species provides the first evidence that HC-Pro is required for virus transmission by a vector other than aphids. Previous experiments (22) demonstrated that transfer of only one wheat curl mite per test plant resulted in WSMV transmission rates of 10% to 37%. Thus, lack of transmission of chimeric viruses by groups of 10 wheat curl mites transferred to each test plant was not likely due to insufficient numbers of wheat curl mites employed in the assay. Also, vector populations increased greater than 10-fold on all but a few test plants, indicating that wheat curl mites survived and reproduced well on test plants. These observations suggest that lack of vector transmission of chimeric WSMV genomes was not because wheat curl mites transferred from source plants subsequently failed to feed on test plants.

Demonstration that chimeric viruses not transmitted by *A. tosichella* were competent for virion assembly (Fig. 4) eliminated lack of encapsidation of chimeric viral RNA by the WSMV-S81 capsid protein as a trivial explanation for loss of vector transmission. Although ELISA indicated that all chimeric WSMV genomes exhibited lower titer relative to S81-SA (Fig. 5), lack of vector transmission was not simply due to reduced virus concentration in infected plants as one vector-transmissible genotype (S81SNOHC1-3) yielded a relative virus titer substantially less than that of four non-vector-transmissible genotypes. A recent study (41) indicated that aphid vector transmission efficiency of purified *Tobacco etch virus* virions was directly correlated with concentration of the HC-Pro protein. Therefore, it is unlikely that the complete lack of wheat curl mite transmission for WSMV constructs bearing HC-Pro from different virus species may be explained by reduced levels of HC-Pro expression (which due to the polyprotein gene expression strategy of WSMV should correlate directly with coat protein expression). Thus, a reduction in vector transmission efficiency (as seen for S81SNOHC1-3), rather than complete abolishment of vector transmission, would be the predicted outcome for constructs with reduced expression of a vector transmission competent HC-Pro. Collectively, these observations indicate that HC-Pro plays an active and qualitative role in mediation of WSMV transmission by the wheat curl mite, rather than an indirect role in which vector transmission competency was solely affected by reduction of virion and/or HC-Pro concentration in source plants.

Of the four constructs having chimeric HC-Pro cistrons with portions derived from ONMV, only S81SNOHC1-3 was transmissible by *A. tosichella* (Table 3). Based on these data, determinants required for vector transmission map to the 5′-proximal half of the WSMV HC-Pro cistron. Sequences required for aphid transmission of potyviruses also map to the 5′-proximal half of HC-Pro (5, 33), although at least one aphid transmission motif (PTK) needed for binding of potyvirus HC-Pro to virions (24) is encoded in the 3′-proximal half. Lack of vector transmission for S81SCOHC2-2 and S81OCSHC4-1 indicated that WSMV sequences both upstream and downstream of the Csp45I site were required. Vector transmission of S81SNOHC1-3 was reduced relative to S81-SA, suggesting that sequences in the 3′-proximal half of HC-Pro may be needed for optimal vector transmission efficiency.

Does WSMV HC-Pro function as a helper component to mediate semipersistent vector transmission? Both aphids and eriophyid mites have piercing-sucking mouthparts. However, the minute size of wheat curl mites (Fig. 3) precludes feeding through artificial membranes. Thus, we were not able to recapitulate the simple but elegant experiments used to demonstrate potyvirus helper component function (19). Within the coat protein of potyviruses, specificity determinants of aphid transmission reside in the amino terminus (16) that includes the conserved DAG motif required for interaction of potyvirus HC-Pro and virions (6). However, the coat protein of WSMV-Sidney 81 does not contain the DAG motif, and among 50+ isolates of WSMV for which coat protein cistron nucleotide sequences are available (48), only El Batán 3 encodes a DAG

motif in the amino terminus. Yeast two-hybrid and in vitro pull-down assays (13) demonstrated that WSMV HC-Pro may interact with itself (presumably to form dimers and/or oligomers), as does potyvirus HC-Pro (17, 21, 27, 41, 49), but no interaction of WSMV HC-Pro with the coat protein or virions was identified (13). Thus, it is possible that WSMV HC-Pro may not interact directly with virions and that formation of a bridge between vector surfaces and virions requires more than a single protein. This is the case for semipersistent aphid transmission of caulimoviruses (17, 38) in which the helper component protein (P2) and P3 protein/virion complexes are acquired sequentially and from different cell types, with the bridge completed by interaction of P2 protein (bound to the vector surface) and P3 protein (previously complexed with virions). Although our data clearly demonstrate that HC-Pro is required for transmission of WSMV by *A. tosichella* and the two protein bridge hypothesis is consistent with available data, the mechanisms by which WSMV acquisition and inoculation are facilitated remain uncertain and in need of further study.

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Mention of proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval to the exclusion of others that also may be suitable.

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