Acquisition and Transmissibility of U.S. Soybean dwarf virus Isolates by the Soybean Aphid, *Aphis glycines*

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Abstract

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Soybean dwarf virus (SbDV) exists as several distinct strains based on symptomatology, vector specificity, and host range. Originally characterized Japanese isolates of SbDV were specifically transmitted by *Aulacorthum solani*. More recently, additional Japanese isolates and endemic U.S. isolates have been shown to be transmitted by several different aphid species. The soybean aphid, *Aphis glycines*, the only aphid that colonizes soybean, has been shown to be a very inefficient vector of some SbDV isolates from Japan and the United States. Trans-

mission experiments have shown that the soybean aphid can transmit certain isolates of SbDV from soybean to soybean and clover species and from clover to clover and soybean with long acquisition and inoculation access periods. Although transmission of SbDV by the soybean aphid is very inefficient, the large soybean aphid populations that develop on soybean may have epidemiological potential to produce serious SbDV-induced yield losses.

Soybean dwarf virus (SbDV) is the causal agent of an economically important disease of soybean (*Glycine max* (L.) Merr.) in Japan (34–37) and a minor disease of other crops elsewhere (21). The causal virus has been transmitted to more than 50 species of forage legumes, pulse crops, and other broadleaf plants representing three families (1,5,6,18,21).

As a member of the Luteoviridae, SbDV is transmitted only by aphids in a circulative, persistent manner. It is phloem-limited and occurs in low concentration in plants (32). The virus consists of several distinct strains based on symptomatology in soybeans, specificity of transmission by aphid species, physiochemical properties, and nucleotide sequence (5,27–30,37). The originally described Japanese soybean strains (SbDV-DS and SbDV-YS), hereinafter called SbDV-D and SbDV-Y, and a subterranean clover red-leaf virus (SCRLV, now synonymous with SbDV) described in Australia (20) were transmitted almost exclusively by *Aulacorthum solani* Kaltenbach. An isolate serologically related to SCRLV found in California (19), and other related isolates found worldwide, were transmitted by *Acyrtosiphon pisum* (Harris) and other aphid species (7,11,16,22,23), and not by *A. solani*. More recently, dwarfing and yellowing strains of SbDV (SbDV-DP and SbDV-YP) have been described from Japan that were transmitted by *Acyrtosiphon pisum* (37). Except for one report (15), all earlier attempts to transmit strains of SbDV by the soybean aphid *Aphis glycines* Matsumora have been unsuccessful (11,32,41). The soybean aphid has been reported to transmit several plant viruses (4,11) including the Indonesian *Soybean dwarf virus* (17). The Indonesian SbDV was shown to be serologically distinct from Japanese SbDV isolates, and no further reports about this virus could be found.

In 2000, the soybean aphid was first reported in the United States in Wisconsin (3), and it has since spread throughout most soybean-producing regions in North America east of the Rocky Mountains. Heavy infestations of soybean aphids often produce severely dwarfed plants with cupped, dark green, or yellowed leaves that resemble the symptoms induced by SbDV (13). Consequently, a link between the presence of *Aphis glycines* and SbDV infection was suspected. In both Japan and the United States, conflicting reports have been published on the ability of *A. glycines* to transmit SbDV (11,15,34,36,38,41). In order to clearly determine possible connections between SbDV reports and the presence of the soybean aphid, extensive transmission experiments were conducted using several U.S. and Japanese isolates of SbDV. We report that endemic strains of SbDV are inefficiently transmitted by *A. glycines* and that length of acquisition and inoculation access periods affect transmission efficiency.

Materials and Methods

Virus isolates, aphid populations, and host plants. Populations of *Aphis glycines* were collected during field surveys in Illinois, Maryland, and Pennsylvania. Aphid identities were confirmed by Doris Lagos and David Voegtlin, University of Illinois, and Susan Halbert, Florida Department of Agriculture, Gainesville. These aphid populations were maintained on soybean ‘Williams 82’ and a glabrous soybean line (PI 547502), from Glen Hartman (IL), in the BSL-3 biosecurity containment facility at Fort Detrick, MD. Cages with aphids were maintained at 24 to 26°C, 16 h D/N with natural and supplemental lighting. Each aphid population was established by nymphal deposit (nymphs separated from adult aphids upon birth and moved to healthy soybean plants for two consecutive transfers) and assayed at each transfer for the presence of contaminating luteoviruses by enzyme-linked immunosorbent assay (ELISA).

Japanese dwarfing and yellowing isolates of SbDV, SbDV-D and SbDV-Y, respectively, were obtained in 1981 from Hokkaido, Japan, and a closely related strain in 1986 from Christchurch, New Zealand. In addition, 13 endemic SbDV isolates from black medic (*Medicago lupulina* L.), *Chenopodium* sp., red clover (*Trifolium pratense* L.), soybean, subterranean clover (*T. subterraneum* L.), and white clover (*T. repens* L.) were obtained from two U.S. mid-

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Atlantic (6,7) and one midwestern state (11). These isolates were used in transmission studies with *A. glycines* (Table 1). Endemic SbDV isolates were characterized as SbDV based on biological and molecular similarities to originally characterized SbDV strains (9,12,31,37). Isolates were routinely transferred with *A. pisum* (pea aphid), *Myzus persicae* (Sulzer) (green peach aphid), and/or *Nearctaphis bakeri* (Cowen) (short-beaked clover aphid) (7,11). Some isolates closely align with the dwarfing genotype of SbDV-D, some with the yellowing genotype SbDV-Y, and some were co-infected with both genotypes (28).

Transmission experiments. Initial transmission experiments were conducted by placing hundreds of non-viruliferous *A. glycines* aphids on detached leaves of young soybean and subterranean clover plants that were infected with Japanese SbDV-D, SbDV-Y, and endemic isolates MD3, MD8, MD9, and VA20 for a 48-h acquisition access period (AAP). Leaves bearing 50 or more aphids then were placed in cylindrical cages over unifoliolate Williams 82 soybean seedlings or clover seedlings for a 48-h inoculation access period (IAP), after which aphids were killed by insecticidal sprays. Inoculated plants were periodically examined for symptoms for 4 weeks before being tested by ELISA and/or reverse transcription-polymerase chain reaction (RT-PCR) for the presence of virus.

Following demonstration of transmission of two endemic SbDV isolates (8), transmission experiments were conducted with additional endemic isolates obtained from red clover, soybeans, and white clover during field surveys from 2005 to 2008 in Maryland and Illinois, using comparable inoculation parameters (Table 1). Known virus/vector combinations (*A. pisum*, *M. persicae*, or *N. bakeri* with endemic SbDV isolates and *A. solani* with Japanese and New Zealand isolates) served as positive controls, and healthy aphids feeding on healthy soybean seedlings served as negative controls with each experiment. Isolates, aphid vectors, and test plants were kept separated in time and space to prevent possible cross-contamination. Each transmission experiment was repeated at least three times with varying transmission parameters.

Short versus long AAP/IAP transmission experiments. Routine acquisition and inoculation access periods for SbDV are 24 or 48 h. However, because of inconsistencies in transmission of SbDV by *A. glycines* utilizing these time parameters, experiments were conducted with lengthened AAPs and IAPs. A series of trials were conducted lengthening the AAP from 48 h to 7 to 14 days and the IAP from 48 h to 10 to 30 days using hundreds of aphids. Non-viruliferous *A. glycines* were allowed to colonize SbDV-infected, symptomatic soybean plants for 7 to 14 days and then permitted to move naturally from the infected plants to healthy soybean seedlings in large cages over a 10 to 30 day IAP. After the IAPs, the plants were sprayed with insecticide to kill the aphids, monitored by visual inspection 30 days for symptom development, and then assayed by ELISA and/or RT-PCR for the presence of the virus.

Following successful transmission of SbDV isolates by *A. glycines* with lengthened AAPs and IAPs, serial passages were attempted within the same inoculated species or between species. At least 25 to 50 or more *A. glycines* were used per seedling with AAPs and IAPs of at least 7 to 10 days. Test plants were observed for symptoms and assayed by ELISA and/or RT-PCR.

Immunological and molecular assays. Confirmation of the presence of SbDV in inoculated plants was accomplished by ELISA, conventional, and/or real-time RT-PCR. ELISA was performed using a commercially available kit according to manufacturer's directions (Agdia).

For conventional RT-PCR, total nucleic acids were extracted from inoculated plants as previously described (40). SbDV RNA was amplified using first-strand cDNA synthesis as follows: 2 µl of total nucleic acid was added to 1× first strand buffer (Invitrogen), 10 mM DTT, 300 µM dNTP mix, 200 nM SbDV 3R (5' GGT ACA CCA CTA TTC AAG AT 3'), and 40 U SuperScript II (Invitrogen). The reaction was incubated at 42°C for 2.5 h. Amplification followed using 1 µl of the first strand reaction with 1× Reaction Buffer (QIAGEN), 200 µM dNTP mix, 200 nM SbDV 4F (5' GAG GGG CTC TGC TCC GAC TCT G 3'), 200 nM SbDV 5R (5' CGT TCA ACC GCC ACC AAT GC 3'), and 0.5 U *Taq* polymerase (QIAGEN). The reaction conditions were: 94°C for 3 min, then 35

Table 1. Nomenclature of *Soybean dwarf virus* isolates included in *Aphis glycines* transmission study

Isolate	Strain(s)	Source host	Location	Contributor
SbDV-D(1981)	D ^a	Soybean	Naganuma, Japan	T. Tamada U51448
SbDV-Y (1981)	Y ^a	Soybean	Naganuma, Japan	T. Tamada L20835
SbDV-NZ (1986)	Y	White clover	Christchurch, New Zealand	J. W. Ashby JF288563
MD1 (1986)	Y	White clover	Fort Detrick, MD	V. D. Damsteegt EU306577
MD2 (1991)	D, Y	White clover	Anne Arundel Co, MD	A. L. Stone EU306578, EU306579
MD3 (1991)	D	<i>Chenopodium</i> sp.	Washington Co., MD	A. L. Stone EU306580
MD6 (2005)	Y	White clover	Prince George's Co., MD	F. E. Gildow JF293280
MD8 (1988)	D, Y	Black medic	Howard Co., MD	A. L. Stone EU306582, EU306583
MD9 (2005)	D	Red clover	Howard Co., MD	A. L. Stone EU306591
MD10 (2006)	Y	White clover	Prince George's Co., MD	V. D. Damsteegt JF288562
MD11 (2006)	D	Soybean	Howard Co., MD	A. L. Stone JF288561
MD16 (2006)	Y	White clover	Prince George's Co., MD	W. L. Schneider JF303084
VA20 (1990)	D, Y	Subterranean clover	Blacksburg, VA	S. A. Tolin EU306589, EU306590
ClAgt1 (2007)	D	Red clover	Champaign Co., IL	L. L. Domier EU419584
ClAgt2 (2007)	D	Red clover	Champaign Co., IL	L. L. Domier EU419583
CL08 (2008)	D	Red clover	Champaign Co., IL	L. L. Domier

^a Originally characterized Japanese dwarfing (D) and yellowing (Y) strains.

cycles of 94°C for 30 s, 57°C for 45 s, and 72°C for 45 s, followed by a final 72°C extension for 5 min.

For real-time RT-PCR reactions, the primer SbDV 1880R (5' CAT TTA TTG GCT ATT ATC TTC C 3') was used to prime reverse transcription reactions using the same conditions described above. Following reverse transcription, 1 µl of first strand reaction with 1× Reaction Buffer (QIAGEN), 200 µM dNTP mix, a total of 6 mM MgCl₂, 1× SmartCycler additive reagent, 1.25 U *Taq* polymerase (QIAGEN), 200 nM SbDV 1880R, 200 nM SbDV 1548F-D (5' GGA YAA GGT TGG TTG YCC TAG GAC GGT 3') or SbDV 1548F-Y (5' GGA YAA GGT TGG TTG GYC CTA GGA CGT T 3'), and 100 nM SbDV 1728 probe (5' FAM-GAT AGC ACC CAG GTT GAT ATG TCT C-BHQ 3'). The reactions were run in a SmartCycler (Cepheid) machine with the following conditions: 95°C for 10 min, then 45 cycles of 95°C for 15 s and 60°C for 1 min. Assay positive result threshold was calculated by the SmartCycler program using 10 standard deviations above background fluorescence.

Persistence of SbDV in aphids following acquisition. The endemic MD16 and VA20 isolates, and Japanese D and Y strains were used to test the amount of SbDV acquired and retained by *A. glycines* and *A. solani*. The *A. glycines* colony of aphids was reared on soybean, and the *A. solani* colony was reared on curly dock (*Rumex crispus* L.). The SbDV-infected source soybeans were first assayed to determine the titers of SbDV. Approximately 100 aphids were placed on detached leaves for a 24 to 48 h AAP. Following the AAP, 15 aphids were placed into 1.5-ml tubes with two leaf disks from a healthy plant and flash frozen using liquid nitrogen. The remaining aphids were transferred to detached leaves of curly dock, nonhost of SbDV, and allowed to feed for 24 to 48 h to clear the gut. After 24 to 48 h, aphids were sampled as before. Total nucleic acids were extracted from the flash frozen aphid samples according to Ali et al. (2), and real-time RT-PCR was performed as described above. Four replicate experiments were conducted using *A. glycines*, and three replicate experiments were conducted using *A. solani*.

Results

Transmission experiments. The transmissibility of 16 SbDV isolates was initially determined using a detached leaf method. Thirteen of these isolates were from the United States (CIAgt1, CIAgt2, CL08, MD1, MD2, MD3, MD6, MD8, MD9, MD11, MD16, and VA20), two from Japan (D and Y), and one from New Zealand (NZ) (Table 1). All transmission experiments using *A.*

glycines with Japanese SbDV-D and NZ from New Zealand were negative (Table 2), but these isolates were efficiently transmitted by *A. solani*. The SbDV-Y strain was inefficiently transmitted (<2%) with lengthened AAP and IAP. None of the U.S. isolates were transmitted by *A. solani*, except for infrequent (<2%) transmission of VA20, some were transmitted by *A. glycines*, and all isolates that were tested were found to be transmissible by *A. pisum* and *M. persicae*. All Illinois isolates were transmitted by *N. bakeri* and all Maryland isolates that were tested with *N. bakeri* were transmitted; however, not all isolates were tested (Table 2).

Less than 5% of the initial *A. glycines* transmissions conducted with CIAgt1, CIAgt2, CL08, MD8, and VA20 utilizing 24 to 48 h AAPs and IAPs were positive, and additional transmission experiments with these isolates were often negative. To increase the efficiency of transmission, aphids were allowed to acquire virus from whole infected plants rather than from detached leaves. Acquisition and inoculation access periods up to 48 h on whole plants did not increase transmission success. When the AAP was lengthened from 48 h to 7 to 14 days and the IAP from 48 h to 21 days on whole plants (not possible with detached leaves), *A. glycines* transmission rates increased (Table 3). Lengthening the AAP beyond 7 days and the IAP beyond 21 days did not increase transmission rates (data not shown).

The endemic isolates were transmitted by *A. glycines* from clover species to soybean, from soybean to soybean, and from soybean to clover species. Serial transmissions of CIAgt1, CIAgt2, and CL08 were successful through four passages using *A. glycines* as the vector on soybean; however, transmissions of MD6, MD10, and VA20 were lost after two to four passages.

Symptoms produced by the different isolates transmitted by *A. glycines* were similar to reported symptoms produced by Japanese SbDV strains transmitted by *A. pisum* (37) or endemic U.S. isolates (7,11) when transmitted by *A. pisum*, *M. persicae*, or *N. bakeri*. Some isolates produced only mild stunting and dark green leaves (D-type), others produced little stunting with characteristic interveinal yellowing (Y-type), and some isolates produced both phenotypes (mixed D and Y) (Table 1).

Persistence of SbDV in aphids following acquisition. A limited number of isolates from the original transmission studies were used to test the amount of SbDV acquired by *A. glycines* and *A. solani*. The original Japanese D was transmissible by *A. solani* but not by *A. glycines*, the original Japanese Y was transmissible by *A. solani* and *A. glycines* (low levels), the MD16 isolate was transmissible by *A. glycines* but not *A. solani*, and the VA20 isolate was

Table 2. Qualitative aphid transmission of U.S. and Japanese *Soybean dwarf virus* isolates by *Aphis glycines* and other reported aphid vectors^a

Isolate/strain	Aphid species				
	<i>A. glycines</i>	<i>Acyrtosiphon pisum</i>	<i>Aulacorthum solani</i>	<i>Myzus persicae</i>	<i>Nearctaphis bakeri</i>
Exotic isolates					
SbDV-D	— ^b	—	+ ^b	—	ND ^b
SbDV-Y	+ ^c	—	+	—	ND
SbDV-NZ	—	—	+	—	ND
Endemic isolates					
MD1	—	+	ND	+	ND
MD2	ND	+	ND	+	+
MD3	—	+	ND	+	ND
MD6	+	+	—	+	+
MD8	+	+	ND	+	ND
MD9	—	+	ND	+	+
MD10	+	+	—	+	ND
MD11	+	+	ND	ND	ND
MD16	+	+	—	+	+
VA20	+	+	+ ^c	+	+
CIAgt1	+	ND	ND	ND	+
CIAgt2	+	ND	ND	ND	+
CL08	+	ND	ND	ND	+

^a More than 25 aphids were used per isolate/plant with each transmission experiment. Not all possible aphid/isolate combinations were completed; most transmission experiments utilized 48 h acquisition access period/48 h inoculation access period.

^b ND = not determined, + indicates positive infection, — indicates negative results.

^c Very infrequent transmission <2%.

transmissible by *A. glycines* and *A. solani* (low levels). Virus titers in source soybean leaves varied with Ct values ranging from 19.7 to 30.1 (data not shown). The PCR reaction limits of detection for D and Y assays approximates 5 fg or a Ct value of 38. The Japanese isolates were acquired by *A. solani*, and the virus was present after 24 to 48 h on the nonhost (Table 4), suggesting the virus had successfully transitioned to the hemocoel and/or accessory salivary gland. The Japanese isolates also were acquired by *A. glycines*, but only the Japanese Y isolate was retained at low levels after the nonhost feed. The endemic isolate MD16 was acquired by both aphid species, but was only retained by *A. glycines*. Endemic VA20 was acquired and retained by both aphid species. Interestingly, the Y component of the VA20 mixed infection was only acquired and retained by *A. glycines* in one of four replicate experiments. The Y component of the VA20 mixed infection exists at lower titers than

the D component in VA20 infected plants (data not shown), and was never acquired by *A. solani*.

Discussion

Soybean dwarf virus disease was described in Japan (33) as having dwarfing and yellowing strains. Both strains were transmitted specifically by *A. solani* (32). Other isolates found in many countries, including the United States, had similar symptomatology, similar hosts, and were transmitted by *A. pisum*, *M. persicae*, *N. bakeri*, and other aphid species but were not transmitted by *A. solani*. In 2001, additional isolates of SbDV were described from Japan (37) that were transmissible by *A. pisum* but not by *A. solani*. In all earlier studies *A. glycines* was always reported as a pest of soybean (the only aphid colonizing soybean) that did not transmit SbDV except for one report (15). This indicated that transmission by *A. glycines* was rare.

The soybean aphid was reported in the United States in 2000 (3,26) and has spread to more than 20 states and Canada. Published reports from surveys for *A. glycines* and possible viruses transmitted by *A. glycines* indicated that it was a vector of several soybean infecting viruses (4,41) but not SbDV. Surveys for soybean diseases in Illinois and Wisconsin (24,25,39) indicated the occurrence of SbDV in some areas, but these occurrences were not linked to disease symptoms or to the presence of *A. glycines*. The majority of reports in the literature have suggested that the *A. glycines*/SbDV vector/virus relationship was a rare possibility at best.

A. glycines were collected from multiple field locations in Illinois, Maryland, and Pennsylvania, and maintained as colonies. These colonies were tested as potential vectors for some endemic isolates of SbDV with the hypothesis that because endemic SbDV isolates had very different vector specificities from the original Japanese SbDV strains (32), they might be transmissible by *A. glycines*. Three independent laboratories in Illinois, Maryland, and Pennsylvania achieved successful transmission with several endemic isolates. However, the level of transmission success was low or completely absent for endemic isolates even though large numbers of aphids were used per seedling. Endemic SbDV isolates were efficiently transmitted at high levels by *A. pisum*, *M. persicae*, and/or *N. bakeri* using 10 to 25 aphids per seedling (Table 2 and data not shown). In contrast, the Japanese and New Zealand isolates of SbDV were highly transmissible by as few as 1 to 2 *A.*

Table 3. Transmission efficiency of *Soybean dwarf virus* (SbDV) isolates VA20 and CIAgt1 by *Aphis glycines* using short and long acquisition access periods (AAP) and inoculation access periods (IAP)

Experiment	SbDV-VA20 ^a		SbDV-CIAgt1 ^b
	AAP 48 h/ IAP 48 h	AAP 7 day+/ IAP 21 day+	AAP 7 day+/ IAP 21 day+
1	1/20 ^c		
2	0/20		
3	4/20		
4	0/20		
5		3/20	
6		4/20	
7		5/20	
8		3/20	
9			4/10
10			7/24
11			13/76
12			1/61
13			2/15
Total	5/80 (6.3%)	18/80 (18.7%)	27/186 (14.5%)

^a Experiments utilized 25 to 50 *A. glycines* per soybean seedling. Reaction based on symptoms and/or enzyme-linked immunosorbent assay (ELISA) results.

^b Experiments utilized 10 to 20 *A. glycines* per soybean seedling. Reaction based on ELISA results.

^c Number positive for SbDV infection/number soybean seedlings tested.

Table 4. Acquisition and retention of *Soybean dwarf virus* (SbDV) endemic isolates MD16, VA20, and Japanese isolates D and Y by *Aphis glycines* and *Aulacorthum solani*

Isolate	Source	Vector	Acquisition and retention ^a		SbDV source aphid Ct values ^b		Nonhost aphid Ct values	
			D	Y	D	Y	D	Y
VA20	Soybean	<i>A. glycines</i>	4/4	1/4	29.1	41.7	32.5	37.4
			3/4	1/4	+/- 2.9	+/- 0.0	+/- 1.3	+/- 0.0
		<i>A. solani</i>	3/3	0/3	22.0	—	27.5	—
			2/3	0/3	+/- 1.3	—	+/- 1.9	—
MD16	Soybean	<i>A. glycines</i>	NA ^c	2/3	NA	31.4	NA	29.8
				2/3		+/- 0.9		+/- 2.1
		<i>A. solani</i>	NA	1/3	NA	28.0	NA	—
				0/3		+/- 0.0		
Japan D	Soybean	<i>A. glycines</i>	3/4	NA	28.2	NA	—	NA
			0/4		+/- 2.7			
		<i>A. solani</i>	2/3	NA	30.9	NA	29.4	NA
			2/3		+/-10.6		+/- 0.5	
Japan Y	Soybean	<i>A. glycines</i>	NA	4/4	NA	31.9	NA	35.5
				3/4		+/- 4.4		+/- 3.5
		<i>A. solani</i>	NA	3/3	NA	33.9	NA	31.1
				2/3		+/- 5.0		+/- 1.1

^a Acquisition and retention values represented by two fractions. The top fraction represents the number of times the aphid acquired the virus from feeding on infected host tissue/total number of experimental repetitions. The bottom fraction represents the number of times the aphid retained the virus after feeding on noninfected nonhost tissue/total number of experimental repetitions.

^b Mean Ct values for all positive results with standard deviations. In cases where only a single sample tested positive, the standard deviation is listed as 0.0. The polymerase chain reaction limits of detection are near 5 fg for both D and Y assays (typical 5 fg = Ct values around 38). Negative reactions indicated by (—).

^c Not applicable.

solani aphids per seedling, but were not transmitted by *A. glycines* or by any of the other aphid species tested.

The inconsistency of transmission of a circulative virus could be due to barriers to virus transmission through the gut into the hemocoel, or to virus accumulation in accessory salivary glands (9,10). The low efficiency and sometimes lack of SbDV transmission by *A. glycines* also could be due to feeding behavior or other unspecified factors. Efforts to improve the efficiency of *A. glycines* transmission of endemic SbDV isolates met with mixed results. Using whole plants as source tissue instead of detached leaves made no difference when the AAP and IAP were 24 to 48 h. Simply increasing the numbers of aphids used was ineffective. The soybean aphid colonizes soybeans very readily with huge numbers per plant, and the use of 100 aphids per unifoliolate seedling was deleterious to the seedlings. However, when longer AAPs of 7 days on young, infected soybeans were followed by 21-day IAPs on seedlings in the second trifoliolate stage or later, *A. glycines* transmission improved dramatically and became more consistent (Table 3). The symptoms produced by the *A. glycines*-transmitted endemic isolates were typical of those produced on plants that had *A. pisum*, *M. persicae*, or *N. bakeri* as the vector. Interestingly, when *A. glycines* was given lengthened AAPs and IAPs on soybean infected with the Japanese Y strain, up to 2% infection rates could be obtained, indicating that if given sufficient interaction with soybeans infected with the Y strain, some transmission would be possible. This pattern was true also for *A. solani* with at least one endemic isolate, VA20 (Table 2).

Two Japanese SbDV strains and two endemic SbDV isolates in soybean were used to test the acquisition of SbDV by vector and nonvector species. The Japanese D isolate is *A. solani* transmissible, the MD16 isolate is *A. glycines* transmissible, and the VA20 and Japanese Y isolates are transmissible by both vectors. Both *A. glycines* and *A. solani* acquired all the isolates tested while feeding on the infected source tissue. However, the retention of the virus through the nonhost feed was correlated to the ability of the aphid to vector the strain used. For example, Japanese D and MD16 were acquired by the nonvector aphid species but never retained. Presumably the virus detected in the aphids feeding on infected host tissue was largely present in the gut, and not necessarily available for transmission. In the nonvector, no virus was detected after the nonhost feed, suggesting that none of the virus crossed the gut barrier. The Japanese Y isolate was acquired and retained by *A. glycines* at low levels, suggesting that a limited amount of the Japanese Y viral particles had crossed the gut barrier into the hemocoel and possibly the accessory salivary gland. However, the rarity of transmission of Japanese Y by *A. glycines* may indicate that successful movement from the gut through the hemocoel into the salivary gland may be restricted. There is no transmission of Japanese Y with short (48 h) AAPs and IAPs, but transmission is possible with long AAPs and IAPs, which further supports this hypothesis.

The endemic isolate VA20 is a mixture of D and Y strains, but following feeding on infected soybeans, only *A. glycines* (the vector) had any traces of the SbDV-Y component. The SbDV-D component was detected in both aphid species from both host feedings. Following the nonhost feeding, both D and Y components of VA20 were only detected in *A. glycines* that had fed on soybeans, but not in the *A. solani* feeding samples. However, the D component of the VA20 mixed infection was acquired and retained by both *A. solani* and *A. glycines*. This contrast is interesting in light of the fact that both aphid species have been known to transmit both components of the mixed infection in previous experiments (Table 2 and data not shown). It should be noted that the acquisition times for the persistence experiments are much shorter than the acquisition times for the transmission efficiency testing. This may affect the results for all the isolates tested. In addition, the ratio of D to Y components in VA20-infected soybeans is regularly biased toward the D component. Perhaps the negative result here is simply a failure to detect marginal amounts of virus.

As seen in Table 2, not all endemic isolates are transmitted by *A. glycines*, and none are transmitted efficiently. In addition, serial

transmission of endemic isolates from one soybean to another could not be maintained indefinitely. Several transmission experiments were lost after two serial transmissions, but others were carried through four transfers. Whether the loss of serial transmission would indicate an impediment to the development of an *A. glycines* vectored epidemic requires further study. The soybean aphid has been shown to colonize red clover but does not survive on white clover. Isolates of SbDV in the Midwest are D-like and known to infect red clover (11,41), while most isolates found in mid-Atlantic states are Y-like or mixed D and Y and found in white clover (7) (Table 1). Isolates obtained from red clover in eastern states (7,28) also were found to be D-like, similar to Midwest isolates. The preference of *A. glycines* for red clover may be significant in the epidemiology of SbDV since it may serve as an alternative host for SbDV, especially SbDV-D.

The soybean aphid has been in soybean fields in the United States for 10 years, and soybean dwarf infected plants have been found at a low percentage each year (24). Although SbDV has the ability to be a devastating disease and an aphid vector that colonizes soybean is now present in the United States, we have not seen evidence of a marked increase in SbDV incidence in soybean production fields (14,38).

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