Aphis glycines as a Vector of Persistently and Nonpersistently Transmitted Viruses and Potential Risks for Soybean and Other Crops

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ABSTRACT

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The recently introduced soybean aphid (*Aphis glycines*), which is widespread in the soybean-growing regions in the United States, is the only aphid able to develop large colonies on soybean. Although its potential as a vector of plant viruses is recognized, reports on virus transmission efficiency by this aphid species are limited. In the present study, we examined the ability of *A. glycines* to transmit several economically important viruses. The results showed that *A. glycines* transmitted the potyviruses *Bean yellow mosaic virus* (BYMV) and *Soybean mosaic virus* from soybean to soybean more efficiently than *Myzus persicae*. However, *M. persicae* transmitted the alfamovirus *Alfalfa mosaic virus* and the potyviruses *Tobacco etch virus* (TEV) and *Tobacco vein mottling virus* (TVMV) from tobacco to tobacco more efficiently than *A. glycines*. This is the first report to demonstrate that the soybean aphid can vector TEV and TVMV, two economically important tobacco viruses. This also is the first report to document successful transmission of BYMV by *A. glycines*. All attempts to transmit the nepovirus *Tobacco ringspot virus* by *A. glycines* were unsuccessful, regardless of the length of the acquisition and inoculation feeding periods. Although the luteovirus *Soybean dwarf virus* (SbDV) was widely distributed in red and white clover in Kentucky, it was not detected in soybean. All transmission experiments of SbDV by *A. glycines* were unsuccessful. A reverse-transcription polymerase chain reaction (RT-PCR) assay was developed to detect SbDV in single aphids using a pair of primers designed to amplify a 372-bp PCR fragment in the coding region of SbDV coat protein. Although *A. glycines* was not a vector of SbDV, the virus was detected in 100% of tested aphids by RT-PCR after a 24- to 48-h virus acquisition access feeding. The practical applications of RT-PCR in detecting persistently transmitted viruses are discussed.

The soybean aphid (*Aphis glycines* Matsumura) was first observed in North America in Wisconsin during the summer of 2000. By the end of the 2000 growing season, it was detected in Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio, and West Virginia. To date, the aphid has spread to more than 20 states and three Canadian provinces. Because of its wide distribution, it is thought that this pest was present in the Midwest for several years before it initially was detected (23).

A. glycines is the only aphid in North America able to develop large colonies on soybean. In addition to the direct damage caused by aphid feeding, the soybean aphid is capable of transmitting the potyvirus *Soybean mosaic virus* (SMV), and the alfamovirus *Alfalfa mosaic virus* (AMV),

two nonpersistently transmitted plant viruses known to naturally infect soybean in the United States (2,17). Wang and Ghabrial (37) demonstrated that *A. glycines* could be a very efficient vector of SMV when aphids were allowed a 1-min acquisition probe. Domier et al. (6) also reported high transmission efficiency of several North American and Asian SMV isolates by the soybean aphid. Although *A. glycines* has been reported elsewhere to transmit several soybean viruses in addition to SMV and AMV including peanut stripe virus (currently classified as a strain of *Bean common mosaic virus*), *Bean yellow mosaic virus*, *Peanut mottle virus*, and *Peanut stunt virus* (15), transmission of these viruses by the soybean aphid has not been documented in the United States.

The nepovirus *Tobacco ringspot virus* (TRSV), the causal agent of bud blight in soybean, is transmitted in nature by the nematode *Xiphinema americanum* (8). It also has been reported to be transmitted at a very low rate by insects and mites, including the aphids *A. gossypii* (Glover), *A. glycines*, and *Myzus persicae* (Sulzer), the grasshopper *Melanopus* sp., the tobacco flea beetle *Epitrix hirtipennis*, *Thrips tabaci* (possibly nymphs), and the spider mites *Tetranychus* sp. (2,31). In soybean, it

is transmitted very efficiently through seed (31). In 2003, a relatively high incidence of TRSV was noted in an *A. glycines*-infested soybean field at the University of Kentucky Experiment Station in Lexington, KY (S. A. Ghabrial, unpublished). This prompted us to examine the transmission of TRSV by the soybean aphid, particularly because TRSV rarely was detected in earlier soybean virus surveys conducted prior to the introduction of the soybean aphid (11). In addition, the recent report that *A. glycines* transmitted TRSV at a low rate (2) added to our interest in the TRSV-*A. glycines* interaction.

The introduction of the soybean-colonizing aphid, *A. glycines*, in the United States raises serious concerns in regard to the spread of persistently transmitted viruses. This is particularly so because the luteovirus *Soybean dwarf virus* (SbDV), which is known to cause significant yield losses in soybean crops in Japan (34), is widespread in forage legumes in the southeastern United States (3). Based on symptoms and host range, the Japanese isolates of SbDV initially were grouped into the yellowing strains (SbDV-Y) and the dwarfing strains (SbDV-D). At present, four distinct phenotypes of SbDV are recognized in Japan, based on symptomatology in infected soybean plants, nucleotide sequences, and vector specificity. These are: the SbDV-YS and SbDV-DS strains, which are transmitted specifically by the foxglove aphid, *Aulacorthum solani* (Kaltenbach); and the SbDV-YP and SbDV-DP strains, which are transmitted by both the pea aphid, *Acyrtosiphon pisum* (Harris) and the clover aphid *Nearctaphis bakeri* (Cowen) (35,36). The SbDV-Y strains infect white clover (*Trifolium repens* L.) but not red clover (*T. pratense* L.). The SbDV-D strains, on the other hand, infect red clover but not white clover (33). The Subterranean clover redleaf virus (SCRLV), which is considered a strain of SbDV, was described in New Zealand and Australia (19,24). Different isolates of SCRLV are transmitted by either *Aulacorthum solani* or *Acyrtosiphon pisum*, but not by both. Interestingly, unlike the Japanese SbDV strains, the *Indonesian soybean dwarf virus* (ISDV), which is widely spread in soybean fields in Indonesia, is transmitted by the soybean aphid in a persistent manner. ISDV, however, is a distinct virus from SbDV, based on serology and cross-protection assays (18).

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Yellowing and dwarfing strains of SbDV have been isolated from forage legumes in the United States and characterized (4). The U.S. isolates are transmitted by *A. pisum*, *Aphis craccivora* (Koch), *M. persicae*, and *N. bakeri* (Cowen), but not by *Aulacorthum solani* (4,13,15). Natural infection of soybean with SbDV is rarely observed and is limited to field borders adjacent to forage legumes, as indicated in a preliminary report from Virginia (9). In a recent disease note, Phibbs et al. (21) reported the first incidence of SbDV on soybean in Wisconsin in *Aphis glycines*-infested soybean fields; however, transmission of SbDV by the soybean aphid was not demonstrated. Except for a recent preliminary report (5), all attempts to transmit SbDV by *A. glycines* have been unsuccessful (15,33). Thus, there is a critical need for documentation of SbDV incidence and distribution in soybean fields and for a better understanding of the vector specificities of the U.S. isolates of SbDV.

The fact that SbDV, like other luteoviruses, occurs in low concentrations in infected plants makes it necessary that sensitive and reliable detection methods are available for studies on incidence and transmission of SbDV by aphid vectors. The reverse-transcription polymerase chain reaction (RT-PCR) methods have been widely used for the detection of RNA plant viruses that occur in low concentrations. Previous studies have shown that viruses can be detected in individual viruliferous aphids using RT-PCR (1,20,26–30,32,37). In addition to evaluating the transmission efficiency of several different plant viruses by *A. glycines*, the objectives of our study included the development of an RT-PCR assay for detecting SbDV in single viruliferous aphids as a means for predicting possible incidence of SbDV in soybean fields. The potential risks of *A. glycines* on the spread of persistently and nonpersistently transmitted viruses in soybean and other crops are discussed.

MATERIALS AND METHODS

Viruses. AMV was isolated from tobacco (*Nicotiana tabacum* L.) in Kentucky, and was maintained in soybean (*Glycine max* cv. Essex) and tobacco (*N. tabacum* cv. Ky-14). Bean yellow mosaic virus (BYMV) was provided by Sue Tolin (Virginia Polytechnic Institute [VPI] and State University) and maintained in soybean (cv. Essex). TRSV was isolated from a soybean field in Kentucky in 2003 and maintained in soybean cv. Essex. SbDV-infected clover and soybean plants were provided by Les Domier (University of Illinois) and Sue Tolin (VPI), respectively. The origin and properties of SMV, Tobacco etch virus (TEV), and Tobacco vein mottling virus (TVMV) have been described previously (37). SMV was maintained in soybean cv. Essex, whereas TEV and TVMV were maintained in tobacco cv. KY 14.

Aphids and transmission tests. *A. glycines* and *M. persicae* were reared and handled as previously described (37). *Acyrtosiphon pisum* was collected from red clover in Princeton, KY (University of Kentucky Research and Education Center) and reared on broad bean (*Vicia faba* L.). Because *A. pisum* originally was collected from red clover in the fields, a representative sample of aphids from the colony were tested by RT-PCR to confirm that the colony was SbDV free prior to conducting the transmission tests. Only apterous late instars were used in all tests. The youngest fully expanded trifoliolate leaves from AMV-, BYMV-, SbDV-, SMV-, or TRSV-infected soybean plants at the V-5 stage (10) or the youngest leaf from AMV-, TEV-, TVMV-, or TRSV-infected tobacco plants in the four- to five-leaf stage were used as virus sources. In all cases, the source leaves were showing symptoms typical of infections with the respective viruses. Because SbDV causes little or no symptoms on clover, the fully expanded youngest leaves were used for RT-PCR tests, and the whole plant (dug from the field and then planted in a pot in a greenhouse) was used as the virus source in aphid transmission tests.

For the transmission tests of BYMV, SMV, AMV, TEV, and TVMV, aphids were collected and kept in glass vials for 2 to 3 h of preacquisition fasting. The starved aphids were allowed a 1-min acquisition probe that was observed under a dissecting microscope. Afterward, they were transferred to test plants: soybean seedlings at the V-1 stage (10) or tobacco seedlings at the two-leaf stage (one aphid/plant). The aphids were allowed to remain on the test plants overnight. After virus inoculation feeding, plants were sprayed with an insecticide and placed in a growth room for 2 to 3 weeks for symptom development. Pertinent double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISAs) were used to confirm virus infection.

For transmission tests of TRSV by *Aphis glycines*, aphids were collected and kept in glass vials for 2 to 3 h of preacquisition fasting. The starved aphids were divided into groups of 5, 10, or 45 to 70 aphids. Each group was allowed 1, 15, or 30 min, 2, 8, or 17 h; or 3 days of acquisition feeding periods on TRSV-infected soybean or tobacco plants. After the acquisition feeding, the aphids were placed on healthy soybean or tobacco plants for 15 min, 1 or 24 h, or 2 or 7 days for inoculation feeding. The plants then were sprayed with an insecticide and placed in a growth chamber for 2 to 3 weeks for symptom development and ELISA testing.

For transmission tests of SbDV by aphids, 300 to 500 aphids were placed on virus-infected clover or soybean for a 24- to 48-h acquisition feeding period, followed by a 24- to 48-h inoculation feeding

period on healthy (confirmed by RT-PCR) clover or soybean seedlings at the V-1 stage (30 to 40 aphids/plant). After virus inoculation feeding, plants were sprayed with an insecticide and placed in a growth chamber for 2 to 3 weeks for symptom development and testing by RT-PCR.

RNA extraction from plants and individual aphids. The extraction of total RNA was carried out as described previously (37) using a commercial RNeasy Mini Kit (Qiagen Inc., Valencia, CA). For plant samples, a leaf disk was ground in liquid nitrogen in a mortar with a pestle, and then mixed with 450 µl of RLT lysis buffer (RNeasy Mini Kit; Qiagen Inc.). The homogenate was transferred into a 1.5-ml microfuge tube and centrifuged for 10 min at 12,000 × g. The supernatant was processed further according to the manufacturer's instructions. The final total RNA was suspended in 50 µl of sterile H₂O. For aphids, individual aphids were placed into a chilled mortar containing liquid nitrogen. As soon as the liquid nitrogen evaporated, the frozen aphid was ground with a pestle, and 200 µl of RLT lysis buffer (RNeasy Mini Kit; Qiagen Inc.) was added into the mortar. The homogenate was mixed further with a pestle. The homogenate was transferred into a 1.5-ml microfuge tube, and the mortar was rinsed twice with 200 µl of RLT buffer each time. The combined homogenate and rinse mixture (a total of 600 µl in RLT buffer) then was centrifuged for 5 min at 12,000 × g. The supernatant was processed further according to the manufacturer's instructions and the final total RNA was suspended in 25 µl of H₂O.

RT-PCR. A pair of primers, a forward primer (SbDV-CPf, 5'-GCGGTTAGC-AATGTCGC-AATAC-3') and a reverse primer (SbDV-CPr, 5'-CATAAGCGA-TGGAACCTGACGA-3'), was designed to prime the amplification of a cDNA fragment of 372 bp in the coding region of SbDV coat protein (CP). The sequences of the forward and reverse primers corresponded to nucleotide positions 3,038 to 3,060 and 3,388 to 3,410, respectively, of the sequence of SbDV strain Tas-1 (GenBank accession number L24049). The SbDV strains sequenced to date have identical sequences to strain Tas-1 in the region covered by the two primers. These are (GenBank accession numbers in parentheses) strain Y (L20835); strain YS, isolate M93-1 (NC_003056); strain YP, isolate M94-1 (AB038148); strain DS, isolate HS-97-8 (AB038149); strain DS, isolate HS-99-4 (AB076050); strain DS, isolate HS-99-2 (AB076049); strain YS, isolate HD-99-4 (AB076048); strain YS, isolate HD-99-3 (AB076047); strain YS, isolate HD-99-3 AB038148; strain DS, isolate HS97-8 (AB038149); strain DP, isolate M96-1 (AB038150); and isolate Wisc3 (DQ145545).

RT-PCR was performed as described previously (37). For leaf samples, two-step

RT-PCR was used. For the RT reaction, 3 µl of total RNA from plants were denatured for 2 min at 72°C and immediately chilled on ice. The samples were added to 17 µl of reaction mixture (4 µl of 5× first-strand buffer, 1 µl of 0.1 M dithiothreitol, 0.5 µl of RNasin [40 U/µl; Promega Corp., Madison, WI], 1 µl of dNTPs [10 mM each], 2 µl of reverse primer [10 pmoles/µl], 0.5 µl of SuperScript II [200 U/µl; GIBCO BRL, Gaithersburg, MD], and 8 µl of H₂O) and incubated for 1 h at 50°C. cDNA (5 µl) was added to 45 µl of PCR reaction mixture (5 µl of 10× PCR buffer containing 25 mM MgCl₂, 1 µl of dNTPs [10 mM each], 3 µl of forward primer [10 pmoles/µl], 2.5 µl of reverse primer [10 pmoles/µl], 0.25 µl of PCR enzyme [Deep Vent DNA polymerase 2 U/µl; New England BioLabs, Beverly, MA], and 33.25 µl of H₂O). Thermal cycling conditions were 2 min at 94°C, followed by 35 cycles (40 cycles for aphid samples) of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, and a final extension of 10 min at 72°C. PCR product (5 µl) was analyzed by agarose gel electrophoresis in 1.2% agarose gels and stained with ethidium bromide.

For aphid samples, a one-step RT-PCR with a commercial kit (SuperScript One-

Step RT-PCR with PLATINUM Taq; Invitrogen Life Technologies, Carlsbad, CA) was used. Total RNA (3 µl; 20 µl for aphid samples) was added to 47 µl (30 µl for aphids) of reaction mixture (25 µl of 2× reaction mix, 2 µl of forward primer [10 pmoles/µl], 2 µl of reverse primer [10 pmoles/µl], 1 µl of RT/PLATINUM Taq Mix, and 17 µl of H₂O [no H₂O for aphid samples]). Thermal cycling conditions were 1 cycle of 30 min at 50°C, 1 cycle of 2 min at 94°C, followed by 40 cycles as described above for the two-step RT-PCR.

To verify the viral origin of the RT-PCR product, it was digested with restriction enzymes *Bsp*EI and *Stu*I. The coding sequence of SbDV CP contains a single *Bsp*EI restriction site at nucleotide position 3,197 and two *Stu*I sites at positions 3,324 and 3,375.

RESULTS

Comparative transmission efficiency of SMV, BYMV, AMV, TEV, and TVMV by *A. glycines* and *M. persicae*. *A. glycines* transmitted the potyviruses BYMV and SMV from soybean to soybean more efficiently than *M. persicae*. *A. glycines* transmitted BYMV and SMV at rates of 10.68 and 36.89%, respectively. The corresponding values for *M. persicae* were 5.98

and 18.64%, respectively (Table 1). However, *M. persicae* transmitted AMV, TEV, and TVMV from tobacco to tobacco more efficiently than *A. glycines*. *M. persicae* transmitted AMV, TEV, and TVMV at rates of 19.33, 48.33, and 52.0%, respectively. The corresponding values for *A. glycines* were 10.67, 7.67, and 12.67%, respectively (Table 1). Furthermore, *M. persicae* transmitted AMV more efficiently than *A. glycines* from soybean to soybean (5.26% compared with 2.07%; Table 1). Interestingly, *A. glycines* transmitted AMV from tobacco to tobacco (10.67% transmission) more efficiently than from soybean to soybean (2.07% transmission).

Attempts to transmit TRSV by *A. glycines*. All attempts to transmit a TRSV isolate, collected from a soybean field in Kentucky in 2003, by *A. glycines* were unsuccessful. No TRSV transmission was obtained regardless of the number of aphids used per plant (from 5 up to 70 aphids per plant), the length of acquisition feeding periods (1 min up to 3 days), or the length of the inoculation feeding periods (15 min up to 7 days). Furthermore, no TRSV transmission was obtained when the virus was acquired from tobacco plants previously inoculated with the same soybean isolate of TRSV (*data not shown*).

Detection of SbDV in red and white clover. RT-PCR was used to detect SbDV in red and white clover collected from three different locations in Kentucky. In total, 44.55% (90/202) of the tested plants were found to be infected with SbDV (Table 2). In the different locations, the proportion of infected plants out of the total tested plants was much higher in red clover (83.3 to 91.7%) than in white clover (23.3 to 38.9%). Most of the clover samples were symptomless in the fields. Soybean leaf samples obtained from neighboring soybean fields in the areas where clover samples were collected were negative for SbDV, as determined by RT-PCR (*data not shown*).

Transmission of SbDV by *A. glycines*. Attempts to transmit SbDV isolates collected from Illinois, Kentucky, and Virginia, by *A. glycines* when the virus was acquired from different SbDV-infected source plants (soybean, red clover, and white clover) were unsuccessful (Table 3). *Acyrtosiphon pisum*, a known vector of the virus, was tested for its ability to transmit the virus, and served as a positive control for the experiments. *A. pisum* transmitted SbDV from either red or white clover to soybean at the rates of 29.4 and 28.6%, respectively (Table 3). *Aphis glycines* failed to transmit the virus from soybean to soybean (Table 3).

RT-PCR. RT-PCR amplification of SbDV RNA from virus-infected plants or from individual aphids generated the expected-size RT-PCR product of 372 bp (Fig. 1). To confirm that the RT-PCR product was of viral origin, it was digested with

Table 1. Comparison between *Aphis glycines* and *Myzus persicae* in transmission efficiency of different viruses^a

Virus	Aphid	Number of infected plants/total tested				Transmission %
		Test 1	Test 2	Test 3	Total	
AMV ^b	<i>A. glycines</i>	1/64	1/57	2/72	4/193	2.07
	<i>M. persicae</i>	4/70	3/52	3/68	10/190	5.26
AMV ^c	<i>A. glycines</i>	6/50	5/50	5/50	16/150	10.67
	<i>M. persicae</i>	9/50	9/50	11/50	29/150	19.33
BYMV ^b	<i>A. glycines</i>	9/74	7/82	9/78	25/234	10.68
	<i>M. persicae</i>	6/79	5/88	4/84	15/251	5.98
SMV ^b	<i>A. glycines</i>	36/93	25/64	46/133	107/290	36.85
	<i>M. persicae</i>	13/66	15/75	24/138	52/279	18.64
TEV ^c	<i>A. glycines</i>	7/100	8/100	8/100	23/300	7.67
	<i>M. persicae</i>	46/100	52/100	47/100	145/300	48.33
TVMV ^c	<i>A. glycines</i>	15/100	12/100	11/100	38/300	12.67
	<i>M. persicae</i>	52/100	57/100	47/100	156/300	52.0

^a Aphids were observed with a dissecting microscope to probe the virus-infected leaves for 1 min, then allowed an overnight inoculation access on test plants (one aphid/plant). Abbreviations: AMV, *Alfalfa mosaic virus*; BYMV, *Bean yellow mosaic virus*; SMV, *Soybean mosaic virus*; TEV, *Tobacco etch virus*; TVMV, *Tobacco vein mottling virus*.

^b Virus was transmitted from soybean to soybean plants.

^c Virus was transmitted from tobacco to tobacco plants.

Table 2. Detection of *Soybean dwarf virus* in clover by reverse-transcription polymerase chain reaction

Sample source ^a	Clover	Number of infected plants/total tested				Infection %
		Test 1	Test 2	Test 3	Total	
Lexington ^b	White clover	4/12	6/12	4/12	14/36	38.9
	Red clover	12/12	10/12	...	22/24	91.7
Murray ^c	White clover	6/20	8/20	6/18	20/58	34.5
Princeton ^d	White clover	4/20	5/20	5/20	14/60	23.3
	Red clover	10/12	10/12	...	20/24	83.3

^a Plants were dug out and planted in pots (one plant/pot) and tested afterward.

^b Plants were collected around greenhouses at the University of Kentucky campus in Lexington.

^c Plants were collected from areas neighboring a soybean field at Murray State University Experiment Station, Murray, KY.

^d Plants were collected from the University of Kentucky Research and Education Center at Princeton.

restriction enzymes *Bsp*EI and *Stu*I. The coding sequence of SbDV CP contains a single *Bsp*EI restriction site at nucleotide position 3,197 and two *Stu*I sites at positions 3,324 and 3,375. As expected, digestion of the RT-PCR product with *Bsp*EI yielded two fragments of 159 and 213 bp (Fig. 2). Likewise, digestion of the RT-PCR product with *Stu*I produced three fragments of 35, 51, and 286 bp (Fig. 2).

SbDV detection in individual *A. glycines*. Although *A. glycines* did not transmit SbDV, the virus was detected by RT-PCR in 100% of the aphids tested after a 24 to 48 h of virus acquisition access (Table 4; Fig. 3A). Moreover, SbDV also was detected in all aphids of a comparable set following a 24- to 48-h inoculation access feeding on healthy soybean plants (Table 4; Fig. 3B). The intensity of the RT-PCR product, as viewed in stained gels, varied somewhat among individual aphids in the same treatment, but there were no apparent differences in band intensity between the two treatments (Fig. 3).

DISCUSSION

Even though the potential of *A. glycines* as a vector of plant viruses is recognized, its impact on soybean production in the United States has not yet been critically assessed. Reports on virus transmission efficiency by this aphid species are limited. The major economically important, aphid-vectored viruses that infect soybean are largely nonpersistently transmitted viruses. In this study, we report the ability of *A. glycines* to transmit AMV, BYMV, and SMV from soybean to soybean, and AMV, TEV, and TVMV from tobacco to tobacco. This study presents the first account of successful transmission of BYMV by the soybean aphid in the United States, even though previous comparable attempts to transmit BYMV were unsuccessful (2). This is also the first report to reveal that *A. glycines* can vector the economically important tobacco viruses TEV and TVMV (25).

A. glycines transmitted BYMV and SMV from soybean to soybean more efficiently than *M. persicae*, but it transmitted AMV, TEV, and TVMV from tobacco to tobacco less efficiently than *M. persicae*. Even though *A. glycines* was found to be a poor vector for some of these viruses under laboratory conditions, its significance as a vector in the field may be considerably greater considering the large population size that potentially could occur in the field. We have shown that *A. glycines* can efficiently transmit nonpersistently transmitted soybean viruses when the aphid briefly probes the infected soybean plant prior to moving to a healthy plant (37). Thus, factors that influence population density, emergence of winged generations, and feeding habits of the individual winged aphids are predicted to have an impact on virus spread by *A. glycines*. If

the numbers of winged aphids increase in a soybean field, this may enhance virus spread within the soybean field as well as pose a significant threat to neighboring crop plants. For example, AMV may spread from soybean fields to neighboring AMV-free tobacco fields. Furthermore, low incidences of AMV, TEV, and TVMV in tobacco fields may be significantly enhanced under conditions where flights of

such alate *A. glycines* from a neighboring soybean field would involve multiple tobacco plant-to-plant hopping, resulting in more efficient spread of these nonpersistently transmitted viruses. In this regard, the finding that *A. glycines* transmitted AMV from tobacco to tobacco more efficiently than from soybean to soybean (this study) is of considerable interest. It is well known that probing plays a major role in

Table 3. Transmission assays of Soybean dwarf virus (SbDV) by *Aphis glycines* and *Acyrtosiphon pisum*^a

Virus source ^b	Test plants ^c	Number of infected plants/total tested			Transmission %
		Test 1	Test 2	Total	
Illinois Red clover	Soybean	0/4	0/4	0/8	0
	White clover	0/6	0/8	0/14	0
Kentucky Red clover White clover	Soybean	0/8	0/9	0/17	0
	Soybean	0/8	0/6	0/14	0
	Soybean**	2/8	2/6	4/14	28.6
	White clover	0/6	0/6	0/12	0
Soybean* Virginia	Soybean	0/10	0/5	0/15	0
Red clover	Soybean	0/8	0/9	0/17	0
	Soybean**	2/9	3/8	5/17	29.4
Soybean	Soybean	0/8	0/4	0/12	0

^a Aphids were given a 24- to 48-h acquisition access on virus-infected source plants, then transferred to test plants (20–30 aphids/plant) for a 24- to 48-h inoculation access.

^b Except as noted by an asterisk (*), plants were naturally infected with SbDV as confirmed by reverse-transcription polymerase chain reaction; * indicates plants infected with SbDV following transmission by *Acyrtosiphon pisum* under laboratory conditions.

^c Except as indicated by asterisks (**), *Aphis glycines* was used for all transmission assays of SbDV; ** indicates *Acyrtosiphon pisum* was used for transmission of SbDV.

Fig. 1. Reverse-transcription polymerase chain reaction (RT-PCR) analysis of total RNA from Soybean dwarf virus (SbDV)-infected plants or individual viruliferous *Aphis glycines*. The primers used for RT-PCR (SbDV-CPf and SbDV-CPr) are specific for the SbDV coat protein coding sequence. The predicted PCR product is 372 bp in size. The RT-PCR products generated with total RNA samples from SbDV-infected and healthy leaves (lanes 2 and 3, respectively) and from single viruliferous and nonviruliferous aphids (lanes 4 and 5, respectively) were subjected to gel electrophoresis on 1% agarose and visualized by ethidium bromide staining. Lane 1: 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD).

Fig. 2. Restriction enzyme analysis of *Soybean dwarf virus* (SbDV) reverse-transcription polymerase chain reaction (RT-PCR) product. Lane 1: 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD); lane 2: *Bsp* EI digestion pattern of the SbDV RT-PCR product showing the predicted two fragments of 159 and 213 bp; lane 3: *Sma* I digestion pattern of the SbDV RT-PCR product with three predicted fragments (35, 53, and 286 bp). The 286-bp fragment was resolved clearly, whereas the 35- and 53-bp fragments were not discernible from each other. Lane 4: undigested RT-PCR product. The RT-PCR and digestion products were fractionated by gel electrophoresis on 1% agarose gel and visualized by ethidium bromide staining.

Table 4. Detection of *Soybean dwarf virus* (SbDV) by reverse-transcription polymerase chain reaction (RT-PCR) in individual *Aphis glycines* aphids

Treatment	Number of virus-carrying aphids/total tested				Detection %
	Test 1	Test 2	Test 3	Total	
After acquisition ^a	8/8	8/8	10/10	26/26	100
After inoculation ^b	12/12	12/12	12/12	36/36	100

^a Aphids were given a 24- to 48-h acquisition access on SbDV-infected white clover, then tested by RT-PCR.

^b Aphids were given a 24- to 48-h acquisition access on SbDV-infected white clover, then transferred onto virus-free soybean for a 24- to 48-h inoculation access. Afterward, they were tested by RT-PCR.

the spread of nonpersistently transmitted viruses in the fields and that, when an aphid lands on a plant following a short- or long-distance flight, it initially will probe the epidermal cells to determine whether the plant is a suitable host rather than begin feeding upon arrival (22). It is these brief probes (usually less than 30 s) used to test the potential food source that are also optimal for acquisition or inoculation of nonpersistently transmitted viruses.

In our laboratory experiments, *A. glycines* failed to transmit TRSV when given different acquisition and inoculation feeding time periods on either soybean or tobacco. Furthermore, the number of aphids used per plant was inconsequential. Our results might not be entirely surprising considering that successful transmission of TRSV by the soybean aphid was reported

in only a single plant out of a total of 48 plants tested under various conditions (2). The relatively high incidence of TRSV in a single soybean field which was infested with *A. glycines* was of concern because TRSV rarely has been detected in soybean in Kentucky. Although the source of TRSV in this specific field was not investigated, it is unlikely, based on our results, that *A. glycines* would be of concern regarding TRSV spread in soybean fields.

The fact that the soybean aphid also is known in Asia to efficiently transmit some luteoviruses in a persistent manner (18) represents a potential risk because a number of luteoviruses (e.g., SbDV and *Bean leaf roll virus*) are endemic in white clover in the soybean-growing regions in the southeastern United States (3). Because luteoviruses are transmitted in a circulative

manner, they have a high level of vector specificity. Each luteovirus (or different strains of the same virus) is efficiently transmitted by only one or a few aphid species (12,13,38). Although SbDV is widespread in clover and its vector species (*Acyrtosiphon pisum* and *Aulacorthum solani*) occur in the United States (3,15, this study), soybean dwarf has not caused major economic problems in soybean production. This possibly is due to the fact that these vectors do not colonize soybean. However, SbDV has different strains and the virus has a high level of vector specificity; therefore, some new strains might emerge and pose a potential threat to soybean production once *A. glycines* is well established.

We demonstrated that SbDV is widely distributed in red and white clover in Kentucky. It is more prevalent in red clover than in white clover (incidence of 83.3 to 91.7% in red clover compared with 23.3 to 38.9% in white clover). All attempts to transmit SbDV by *Aphis glycines* were unsuccessful regardless of whether the virus source was SbDV-infected clover or soybean (including soybean plants naturally infected with a Virginia isolate of SbDV). These results are consistent with those of Harrison et al. (15). In apparent contrast, Damsteegt et al. (5), in a preliminary report, presented evidence that *A. glycines* transmitted two eastern U.S. isolates of SbDV. It is possible that changes in the transmission phenotypes of SbDV isolates, perhaps as a result of mutations in the viral determinants of aphid transmission specificity, most likely the 54-kDa readthrough domain, may render such viral variants transmissible via *A. glycines* (35). The preliminary report by Domier et al. (7) is of interest in this regard because these authors showed a high level of amino acid substitutions targeted to the readthrough domain of a Wisconsin soybean isolate of SbDV compared with a dwarfing Japanese SbDV isolate. Whereas the latter is not transmissible by *A. glycines* (known to be most efficiently transmitted by *Acyrtosiphon pisum*), the Wisconsin isolate was obtained from a soybean field heavily infested with the soybean aphid (21). Unfortunately, no *Aphis glycines* transmission assays were carried out with the Wisconsin SbDV isolates.

RT-PCR is an extremely sensitive technique and has been used widely to detect plant viruses that occur at relatively low concentrations in their host plants or that are unevenly distributed in the plants (16). It also has been used to detect several persistently and nonpersistently transmitted viruses in single aphids (1,20,26–30,32,37). All these studies emphasized that virus detection in aphids by RT-PCR has a potential application in monitoring virus incidence in the fields. For nonpersistently transmitted viruses, Wang and Ghabrial (37) noted that the rate of SMV

Fig. 3. Reverse-transcription polymerase chain reaction (RT-PCR) products generated with total RNA from individual aphids after a 24- to 48-h acquisition access periods on *Soybean dwarf virus* (SbDV)-infected white clover plants. **A**, Aphids with a 24- to 48-h acquisition access periods on SbDV-infected white clover plants. Lanes 1 and 20: 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD); lanes 2 to 17: samples from individual viruliferous aphids; lane 18: a sample from nonviruliferous aphid; lane 19: a sample from an SbDV-infected clover leaf used for virus acquisition feed. **B**, Aphids with a 24- to 48-h acquisition access periods to SbDV-infected white clover plants, followed by a 24- to 48-h inoculation access on healthy soybean plants. Lanes 1 and 20: 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD); lanes 2 to 17: samples from individual viruliferous aphids; lane 18: a sample from a nonviruliferous aphid; lane 19: a sample from an SbDV-infected clover leaf used for virus acquisition feed. The RT-PCR products were visualized by electrophoretic separation on 1% agarose gels and ethidium bromide staining.

detection by RT-PCR in aphids that were allowed a brief acquisition probe was similar to the transmission rate and proposed to collect and test noncolonizing aphids to predict the prevalence of these viruses in the fields. The use of RT-PCR for the detection of targeted persistently and nonpersistently transmitted viruses (1,14,26, 29,37, this study) in infected soybean plants as well as in single aphids collected from suction traps should provide a reliable and highly sensitive means for monitoring virus incidence in soybean fields infested with the soybean aphid.

An RT-PCR assay was developed to detect SbDV in single soybean aphids using a pair of primers designed to amplify a 372-bp PCR fragment in the coding region of SbDV CP. Although *A. glycines* was not a vector of SbDV in our experiments, the virus was detected in 100% of the aphids tested by RT-PCR after a 24- to 48-h virus acquisition feeding period, and even after such aphids were given an additional 24- to 48-h inoculation feeding period on healthy soybean plants. Because of the potential generation of *A. glycines*–

transmissible SbDV variants, positive RT-PCR results with aphids collected from infested soybean fields or suction traps should serve as an important warning to monitor SbDV incidence in the respective soybean fields.

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