Incidence of Soybean dwarf virus and Identification of Potential Vectors in Illinois

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ABSTRACT

Soybean dwarf virus (SbDV), which causes an important disease of soybeans in Japan, is persistently transmitted by aphids and is endemic in forage legumes in the United States. To determine the incidence of SbDV in Illinois, we collected clovers and forage legumes in a total of 49 Illinois counties in 2001 and 2002 and tested them for the presence of SbDV by reverse-transcription–polymerase chain reaction. SbDV was detected in 43% of red clover (Trifolium pratense), 10% of white clover (T. repens), and 3% of yellow sweet clover (Melilotus officinalis) plant samples. The dwarfing strain (SbDV-D) was the predominant strain detected in Illinois. In 2000, Aphis glycines, an aphid species that colonizes soybeans, was reported for the first time in North America. To determine whether A. glycines or aphid species found colonizing clover were vectors of SbDV, transmission studies were conducted. Aphids of the species Nearctaphis bakeri reproducibly vectored SbDV among red clovers, and from red clover to soybean. A. glycines did not transmit SbDV; neither did two other clover-infecting aphid species, Acyrthosiphon pisum and Therioaphis trifolii.

Soybean dwarf virus (SbDV), a member of the Luteoviridae, was first identified in association with outbreaks of dwarfed soybean (Glycine max L.) plants that had severe yield losses in northern Japan in 1969 (29). Subsequently, similar viruses were identified in Australia, Ethiopia, Iran, New Zealand, Syria, and the United States (1,20,22-25,33). In northern regions of Japan, SbDV is one of the most important virus diseases of soybean (15). Yield losses from SbDV have been shown to be linearly correlated with percent SbDV infection in soybean fields, with a 50% infection causing a 40% yield loss that results from a reduced number of pods set (2,28). Due to the persistent nature of its transmission, SbDV is transmitted efficiently only by colonizing aphids (26). In the United States, SbDV has been detected primarily in clover plants and rarely infects soybean, presumably because of the absence of aphid vectors that colonize soybean (9,10).

In Japan, the polyphagous Aulacorthum solani (Kaltenbach) is the principal vector of SbDV (15). In northern Japan, A. solani is holocyclic, and its eggs overwinter on red clover (Trifolium pratense L.) and white clover (T. repens L.) plants, many of which are infected with SbDV (2,15). In the spring, viruliferous winged aphids develop on these hosts and fly to soybean, where feeding of viruliferous aphids leads to SbDV infections. The disease is spread in the field by parthenogenic apterous aphids throughout the summer, until sexual generations emerge again in autumn and migrate back to clover to lay eggs and complete their life cycle (2,15).

Many species from several plant families are susceptible to SbDV, but the most common hosts of SbDV are members of the Fabaceae (4,8,30). Based on the symptoms they produce in soybean, SbDV isolates have been divided into two strains: dwarfing (SbDV-D) and yellowing (SbDV-Y) (27). SbDV-D symptoms in soybean plants include shortened internodes and petioles and dark colored, brittle leaves that curl downward. SbDV-Y causes less stunting, but the symptoms tend to be more severe and include interveinal chlorosis, thickened and brittle mature leaves, and leaflets that do not fully develop and become rugose (30). Dwarfing and yellowing strains of SbDV show differences in host range. Dwarfing strains infect red clover but not white clover, while yellowing strains infect white clover but not red clover (8,26).

Like other members of the Luteoviridae, SbDV isolates can show differential transmission by different aphid species, which has been used to further subdivide the dwarfing and yellowing strains (32). SbDV-DS strains are transmitted by A. solani, and SbDV-YP strains are transmitted by Acyrthosiphon pisum (Harris) and Nearctaphis bakeri (Cowen). Similarly, SbDV-Y strains are transmitted by A. solani, and SbDV-YP strains are transmitted by Acyrthosiphon pisum, N. bakeri, and very rarely by Aphis glycines Matsuura (16). SbDV isolates from different geographic regions have been used to further subdivide the dwarfing and yellowing strains. Japanese SbDV isolates were transmitted by A. solani, Acyrthosiphon pisum, and N. bakeri, but not by Myzus persicae (Sulzer) or Aphis craccivora Koch (16,26). In New Zealand and Tasmania, Acyrthosiphon pisum, Aulacorthum solani, and Macrosiphum euphorbiae (Thomas) vectored white-clover infecting isolates of SbDV, but as in Japan, M. persicae and Aphis craccivora did not transmit SbDV (19). In the United States, SbDV isolates from white clover were transmitted by both Acyrthosiphon pisum and M. persicae, but not by Aulacorthum solani (9,12,19).

This specificity of transmission is engendered by interactions between aphid membrane barriers and virus proteins. Gildow et al. (12) examined the movement of transmissible and nontransmissible SbDV isolates through M. persicae and observed that a nontransmissible SbDV isolate could not penetrate the salivary basal lamina of M. persicae. The salivary basal plasmalemma, the cell layer adjacent to the basal lamina, served as the barrier for transmission for an isolate that was not transmitted by A. solani. SbDV-D and SbDV-Y strains share 83% amino acid sequence identity (32). The greatest sequence variability between the two strains of SbDV is found in the C-terminal half of the coat protein readthrough domain and the 3′ noncoding region (32). Yet amino acid sequence variation in the N-terminal region of the readthrough domain has been reported to be related to specificity of aphid transmission of SbDV (31).

Aphis glycines was identified in the United States for the first time in 2000 (13) and subsequently has spread throughout much of the soybean growing region of North America (17). The introduction of A. glycines, an aphid species that colonizes soybean plants, has the potential to increase the incidence in North America of persistently and nonpersistently transmitted viruses in soybean. A. glycines has

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been reported to transmit several viruses, including Alfalfa mosaic virus, Bean common mosaic virus, Bean yellow mosaic virus, Cucumber mosaic virus, Indonesian soybean dwarf virus (ISDV), Peanut stripe virus, Peanut stunt virus, Soybean mosaic virus (SMV), and Tobacco ringspot virus (3,7,14,18). ISDV is another member of the Luteoviridae that causes symptoms similar to SbDV in soybean but is serologically distinct from SbDV (18). While in one instance A. glycines was reported to transmit SbDV rarely (15), other reports have found no transmission of Japanese isolates of SbDV by A. glycines (28,29).

This is the first study of SbDV in Illinois. Our objectives were to determine the distribution of SbDV in Illinois in forage legumes that border soybean fields, determine the prevalent strain(s) of the virus, and identify potential vectors of the virus in Illinois.

MATERIALS AND METHODS
Reverse transcription–polymerase chain reaction (RT-PCR). SbDV infections were detected by RT-PCR. RNA was extracted from leaf tissue homogenized in TRIZOL reagent (Invitrogen Corporation, Carlsbad, CA) following the manufacturer’s recommendations. For RT-PCR, the SuperScript One Step RT-PCR System (Invitrogen) was used, and reactions were performed in PTC-100 Programmable Thermal Controllers (MJ Research, Inc., Watertown, MA). Reactions consisted of 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. For most RT-PCR assays, SbDV-3248F (GGGAATATGAC-CTTCTGGGCGCGTCT) and SbDV-3529R (G GCCATGATACCAGTGAAGACC) were used as sources of inoculum for transmission studies described below. In 2002, leaf tissue from clover plants was collected from an average of two locations in 36 Illinois counties. All plants were analyzed for SbDV infection using RT-PCR. In 2001, a subset of plants negative for SbDV infection with primers SbDV-3248F and SbDV-3529R were restested by RT-PCR using Dicot-Luteo 1 F and Dicot-Luteo 1 R. In 2002, the clover samples were analyzed only by RT-PCR with SbDV-3248F and SbDV-3529R.

Aphid colonies. Virus-free aphid colonies were established for five aphid species collected from Illinois: Aphis craccivora, A. glycines, Acrystosiphon pismum, N. bakeri, and Therorips trifolii (Monell). Aphids were identified as described by Blackman and Eastop (3), and identifications were confirmed by David Voegtlin of the Illinois Natural History Survey. To clean the field-collected aphids of persistently transmitted viruses, they were placed on greenhouse-grown healthy red clover leaves and new-borne nymphs were immediately transferred to leaves of healthy plants. Aphis craccivora was reared on healthy Vicia faba L. cv. Improved Long Pod. Aphis glycines was reared on healthy soybean cv. Williams 82. Acrystosiphon pismum, N. bakeri, and T. trifolii were reared on healthy red clover. All colonies were maintained in cylindrical acrylic cages in growth chambers maintained at 23°C and 17-h day length.

Transmission of SbDV by aphids. SbDV-D isolates were used for transmission studies because they were the most prevalent in Illinois. Five SbDV-positive clover samples, two from Champaign County and one each from Coles, Camberland, and Douglas counties in Illinois, were used as source tissue in initial aphid transmission tests for SbDV-D. Preliminary experiments were performed to determine if Acrystosiphon pismum (a potential positive control for SbDV-D transmission) or Aphis glycines transmitted SbDV-D when soybean seedlings were infested with multiple aphids. Approximately 20 aphids of Acrystosiphon pismum and Aphis glycines were placed on detached leaves from red clover infected with each of the five SbDV isolates in petri dishes containing moistened filter paper and given an acquisition access period of 3 h. The sequences of primers SbDV-5406 and SbDV-5493 are shown above an alignment of the corresponding 3′ NCR that was diagnostically variable between dwarfing and yellowing strains. RT-PCR reactions were performed as above with an annealing temperature of 55°C.

Incidence of SbDV infection. Clover and other plants were collected from ditches and field borders during the growing seasons of 2001 and 2002 from an average of two locations per county. In 2001, entire plants were dug up from 35 counties, brought back to the greenhouse, and replanted in pots. Plants that tested positive for SbDV by RT-PCR were used as sources of inoculum for transmission studies described below. In 2002, leaf tissue from clover plants was collected from an average of two locations in 36 Illinois counties. All plants were analyzed for SbDV infection using RT-PCR. In 2001, a subset of plants negative for SbDV infection with primers SbDV-3248F and SbDV-3529R were restested by RT-PCR using Dicot-Luteo 1 F and Dicot-Luteo 1 R. In 2002, the clover samples were analyzed only by RT-PCR with SbDV-3248F and SbDV-3529R.

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Fig. 1. Differentiation of Soybean dwarf virus (SbDV) strains. A, The sequences of primers SbDV-5406 and SbDV-5493 are shown above an alignment of the corresponding 3′ noncoding regions of SbDV-D and SbDV-Y strains. Amplifications of SbDV-D isolates are predicted to yield 110-bp fragments, while SbDV-Y isolates should produce fragments of 151 bp. B, Analysis of 10 Illinois SbDV isolates from red clover with primers SbDV-5406 and SbDV-5493. All samples analyzed produced an 110-bp fragment, the size predicted for dwarfing isolates of SbDV.
A final transmission study to test the ability of *Aphis glycines* to vector ShDV from infected to healthy soybean plants was conducted. Two ShDV-infected soybean plants were placed in separate 25 × 75 cm cages and infested with *A. glycines*. After 2 weeks, when the aphids densely colonized the ShDV-D–infected plants, pots containing five to six healthy soybean plants were introduced into the cages. After 4 weeks, when the introduced plants were heavily infested with aphids, all plants were tested by RT-PCR for ShDV infection.

**RESULTS**

**Incidence of ShDV in Illinois.** In 2001, 120 clover and other plants were collected from 35 Illinois counties and tested for ShDV (Fig. 2). Enzyme-linked immunosorbent assay using ShDV-specific monoclonal antibodies (11) was evaluated for detection of ShDV infections, but because of high backgrounds in clover samples (data not shown), RT-PCR was deemed more reliable and used to detect the presence of ShDV. Of the clover plants tested, 64 were red clover, 19 of which tested positive for ShDV by RT-PCR (Table 1). No ShDV infections were found in yellow sweet clover (*Melilotus officinalis* L.), black medic (*Medicago lupulina* L.), or white clover. To determine whether plants negative in RT-PCR with ShDV-3248F and ShDV-3529R were infected with other luteoviruses, the PCR-negative samples were reanalyzed by RT-PCR using degenerate primers *Dicot-Luteo* 1F and *Dicot-Luteo* 1R, which were designed from the sequences of dicot-encoding luteoviruses. All samples that were negative with the ShDV-specific primers (ShDV-3248F and ShDV-3529R) were tested with these degenerate primers and found to be negative for luteoviruses.

**Table 1. Incidence of Soybean dwarf virus (ShDV) in forage legumes collected in Illinois in 2001 and 2002 as determined by reverse transcription–polymerase chain reaction (RT-PCR)**

<table>
<thead>
<tr>
<th>Plant type</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red clover</td>
<td>19/64&lt;sup&gt;4&lt;/sup&gt;</td>
<td>50/96</td>
</tr>
<tr>
<td>White clover</td>
<td>0/11</td>
<td>3/20</td>
</tr>
<tr>
<td>Yellow sweet clover</td>
<td>0/14</td>
<td>1/17</td>
</tr>
<tr>
<td>Black medic</td>
<td>0/17</td>
<td>0/20</td>
</tr>
<tr>
<td><em>Oxalis stricta</em></td>
<td>0/4</td>
<td>0/17</td>
</tr>
<tr>
<td>Other clover species</td>
<td>0/10</td>
<td>0/5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19/120</strong></td>
<td><strong>54/175</strong></td>
</tr>
</tbody>
</table>

<sup>4</sup> Number of plants positive by RT-PCR/number tested by RT-PCR.
Aphid transmission of SbDV. In initial transmission studies using 20 aphids per plant, neither *Aphis glycines* (0 of 99 plants) nor *Acrithosiphon pismum* (0 of 87 plants) transmitted SbDV-D from red clover to soybean. Since *A. pismum* was expected to serve as a positive control for the remaining transmission studies, three other aphid species found colonizing clover (*Aphis craccivora*, *N. bakeri*, and *T. trifolii*), along with *Aphis glycines* and *Acrithosiphon pismum*, were tested for their abilities to vector SbDV-D. In transmission experiments using approximately 100 aphids per pot of red clover seedlings, two of three 10-plant samples from one pot inoculated with SbDV using *Aphis craccivora* were positive for SbDV, and one 10-plant sample from each of two pots inoculated with SbDV using *N. bakeri* was positive. SbDV was not transmitted by *Aphis glycines*, *Acrithosiphon pismum*, or *T. trifolii* in this experiment. When the experiment was repeated with fewer clover plants and a shorter IAP, none of the aphid species transmitted SbDV from red clover to red clover. When the same five aphid species were tested for their abilities to vector SbDV-D from red clover to soybean using approximately 100 aphids of each species per pot of soybean seedlings, only *N. bakeri* transmitted SbDV.

Since *Aphis craccivora* and *N. bakeri* transmitted SbDV in preliminary tests, experiments were conducted to test the efficiencies of SbDV-D transmission from red clover to soybean by the two aphid species at 25 and 30°C. *Aphis glycines* was included in the trial as an additional test of its vector competence. When one aphid per plant was used, transmission of SbDV occurred only with *N. bakeri* at 30°C (Table 2). When the number of aphids per plant was increased to seven, again only *N. bakeri* transmitted SbDV-D from red clover to soybean (Table 2). The infected soybean plants showed symptoms characteristic of SbDV-D that included dwarfing due to shortened internodes and smaller, thicker, darker green and downward curled leaves, as described by Chang (6). Because of the low efficiency of transmission, it was not possible to draw conclusions regarding the influence of temperature on transmission. In the final transmission study, where *Aphis glycines* was allowed to completely colonize SbDV-D-infected soybean plants and then migrate to healthy soybean plants, no virus transmission was detected as determined by RT-PCR.

**DISCUSSION**

The dwarfing strains of SbDV were the most common in clovers in Illinois during 2001 and 2002. This conclusion was supported by RT-PCR results and by the predominance of infection of red clover, which is commonly infected by SbDV-D in both Japan and the United States but rarely infected by SbDV-Y (8,19,27). When only the SbDV-positive RT-PCR results are considered (Table 1), the detection of SbDV-infected red clover in Illinois increased from 30% in 2001 to 52% in 2002, with positive samples detected in eight additional Illinois counties (Fig. 2). While SbDV incidence may have increased in Illinois from 2001 to 2002, the larger area sampled and/or the improved sampling and detection techniques used in 2002 may have contributed to the increase in the number of positive plant samples.

In the present study, less than 10% of the white clover plants sampled were infected with SbDV. In contrast, Damsteegt et al. (9) detected SbDV in 47% of white clover samples from eastern and southeastern states. Several factors may be responsible for these contrasting results. The surveys in the eastern United States were conducted 5 years earlier in white clover pastures, whereas clovers in the current study were found in ditches surrounding soybean fields. Virus infection may be more likely in clover pastures where the plants are in a higher density and cover a larger area, conditions that favor disease spread. In addition, vegetative reproduction of white clover in pastures over multiple years can result in growth of several diseased plants originating from one infected parent. The dissimilar results also may be attributed to the different environments of the regions of the United States in which the studies were conducted, or to the use of different detection methodologies. Additional studies with different sampling protocols and possibly larger numbers of samples will need to be conducted to identify the source of the differences in incidences of SbDV-Y isolates in the two studies.

*Acrithosiphon pismum*, *Aphis craccivora*, *N. bakeri*, and *T. trifolii* were found colonizing clovers in Illinois and tested for their ability to transmit SbDV. *Acrithosiphon pismum* has been reported to vector U.S. isolates of SbDV (10). *Aphis craccivora* transmits at least 40 viruses, including the luteoviruses BLRV, BWYV, CPSSDV, and GRAY (5). In previous transmission studies in Japan and New Zealand, *A. craccivora* failed to transmit selected isolates of SbDV to soybean plants. In the present study, *A. craccivora* transmitted SbDV-D to soybean plants in 2001 and 2002. Further work is needed to determine which factors were responsible for the differences between the studies in Japan and the United States and to determine whether these factors are related to differences in the luteoviruses present in the two regions.

**Table 2. Transmission of Soybean dwarf virus (SbDV-D) from red clover to soybean by three aphid species.**

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>Transmission (one aphid per plant)</th>
<th>Transmission (seven aphids per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td><em>A. craccivora</em></td>
<td>0/24</td>
<td>0/22</td>
</tr>
<tr>
<td><em>A. glycines</em></td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td><em>N. bakeri</em></td>
<td>0/24</td>
<td>1/20</td>
</tr>
</tbody>
</table>

* Number of infected plants/number of inoculated plants.
ShDV isolates (26,33). *N. bakeri* colonizes red clover, but unlike *A. craccivora*, its potential as an aphid vector has not been studied in detail. However, it has been reported to transmit viruses in several families and was recently reported as a vector of ShDV-DP (3,16) and ShDV-YP (21). *T. trifoli* also has been reported to transmit legume-infecting viruses (3), but has not been reported to transmit ShDV. In our studies, *N. bakeri* transmitted ShDV in a few instances, but at very low efficiencies. *A. craccivora* transmitted ShDV in initial trials with large numbers of aphids (ca. 100), but did not vector ShDV when one or seven aphids were used per plant. These results may reflect events taking place in the field, i.e., low transmission rates by aphid species that colonize in large numbers, or there may be other aphid clones from these species that transmit ShDV-D more efficiently. The low transmission efficiencies observed in these studies also may have been due to low virus titers in the clover plants used as ShDV sources. It is also possible that other aphid species that were not collected and tested transmit ShDV at higher efficiencies.

As reported previously for Japanese ShDV isolates (28,29), we found no evidence of transmission of ShDV by *Aphis glycines*. Hence, *A. glycines* is unlikely to be an important vector of ShDV in Illinois. This is supported by the observations that none of the more than 1,000 soybean plants that were tested for ShDV in 2001 and 2002 were positive for ShDV infection despite high *A. glycines* populations (data not shown). *N. bakeri*, which was found heavily colonizing forage legumes close to soybean fields, was capable of transmitting ShDV-D to soybean plants, but only at low levels. In addition, *N. bakeri* produced very few nymphs on soybean plants that survived only a few days. These factors may explain why transmission of ShDV by *N. bakeri* has not become a significant threat to soybean production in Illinois.

**ACKNOWLEDGMENTS**

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