Transmission of Sorghum Stunt Mosaic Rhabdovirus by the Leafhopper Vector, *Graminella sonora* (Homoptera: Cicadellidae)

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ABSTRACT

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The transmission parameters of sorghum stunt mosaic rhabdovirus (SSMV) by the leafhopper *Graminella sonora* were determined. The minimum acquisition and inoculation times were 6 and 1 h, respectively, while 100% transmission was obtained from a 48-h acquisition access or a 24-h inoculation access period. Transmission efficiency was highest at temperatures of 24 to 36°C. The minimum latent period prior to transmission was 9 days at 30°C, with an 11-day incubation period necessary for 100% transmission. After a 14-day incubation period at 16°C, no transmission was obtained; however, doubling the incubation period to 28 days gave high levels of transmission. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) of individual leafhoppers held at 16°C for a 14-day incubation period detected no virus in leafhoppers, whereas over 50% of the leafhoppers had detectable virus after a 28-day incubation period. *Graminella nigrifrons* and *Peregrinus maidis* were not able to transmit SSMV.

Rhabdoviruses cause serious plant disease outbreaks in many parts of the world. Plant rhabdoviruses are composed of bacilliform or bullet-shaped enveloped particles with a single molecule of single-stranded RNA. These viruses are transmitted by a variety of invertebrate vectors, including leafhoppers, plant hoppers, aphids, a lacebug, and a mite, to monocots and dicots (9). The interaction of rhabdoviruses with their leafhopper and plant hopper vectors is highly specific. Generally, one to two vector species of the same genus transmit a single rhabdovirus, and that virus also replicates within its insect vector (6).

Sorghum stunt mosaic rhabdovirus (SSMV), vectored by the leafhopper *Graminella sonora* (Ball), was first reported infecting monocotyledons in California in 1981 (10). The physiochemical aspects of the virus have been characterized (5), but little else is known about SSMV. Knowledge of the transmission biology of this virus is essential to understanding its epidemiology.

Detailed information on the transmission parameters of plant rhabdoviruses to monocots is limited to maize mosaic virus (MMV) (1) transmitted by the plant hopper *Peregrinus maidis* Ashmead, rice transi-

tory yellowing virus (RTYV) (8) transmitted by several *Nephotettix* spp. (leafhoppers), and wheat striate mosaic virus (WSMV) (12) transmitted by two species of the leafhopper *Elymana*. All three viruses can be transmitted efficiently with an acquisition access period of 4 h. However, the incubation or latent period after acquisition before virus can be transmitted is variable. Incubation periods of 8 to 34 days, 4 to 24 days, and 9 to 29 days have been reported for RTYV (2), WSMV (12), and MMV (1), respectively. Incubation periods of 8 to 10 days, 11 to 24 days, and 5 to 35 days were reported for barley yellow striate mosaic virus (BYSMV) transmitted by *Laodelphax* and *Javesella* spp. of plant hoppers (4), cereal chlorotic mottle virus (CCMV) transmitted by the leafhopper *Nesoclutha pallida* (Evans) (7), and winter wheat mosaic virus (WWMV) transmitted by two species of the leafhopper *Psamotettix* (6), respectively. The temperature at which the insects are held during the incubation period has a significant effect on the efficiency of transmission and the length of the incubation period of virus in the hopper vectors. WSMV transmission efficiency at 32°C was 20% higher than at 24°C (13). The RTYV incubation period tripled from 6 days at 36°C to 18 days at 24°C (8). In this paper, we describe the characteristics of transmission of SSMV by the leafhopper vector *G. sonora*.

MATERIALS AND METHODS

Maintenance of virus and insects. An isolate of SSMV obtained from naturally infected *Zea mays* L. (5) was maintained in sweet corn, *Z. mays* cv. Golden Bantam, at

30°C by successive transfers using *G. sonora*. Infected plants were used as source plants approximately 2 weeks after initial symptom expression. Healthy *G. sonora* were reared in California on a mixed culture of virus-free oats (*Avena sativa* L. cv. Cal Red Oats) and maize in a growth chamber at 30°C with a 16:8 (L:D) photoperiod, while *Graminella nigrifrons* (Forbes) and *P. maidis* were reared in Ohio on maize (14). The origin of the leafhopper colonies has been previously reported (11). Transmission tests using *G. sonora* were carried out in California, and tests with *G. nigrifrons* and *P. maidis* were done in Ohio.

Minimum acquisition, inoculation, and transmission period tests. Non-viruliferous adult *G. sonora* leafhoppers were given an acquisition access period (AAP) on detached corn leaves at 25°C in a plastic box under fluorescent lights. The leafhoppers were then caged on healthy corn for an inoculation access period (IAP) in a growth chamber at 30°C (unless otherwise stated) with a 16:8 photoperiod. Five adult leafhoppers per plant were allowed to feed on each of 10 test plants for each of three replicates in each experiment. Leafhoppers were removed, and the plants were sprayed with resmethrin insecticide (Whitmire Research Laboratories, St. Louis, MO) to kill any remaining insects. The plants were maintained in a leafhopper-free greenhouse at least 1 month to monitor symptom development, and unless otherwise stated, plants were tested for virus by enzyme-linked immunosorbent assay (ELISA).

To determine the minimum AAP for SSMV, *G. sonora* leafhoppers were starved for 1 h, then allowed to feed on detached infected maize leaves for 3, 6, 12, 24, or 48 h. The leafhoppers were then transferred to healthy sweet corn test plants for 14 days. The minimum IAP required for transmission of SSMV was determined by allowing *G. sonora* leafhoppers to feed on infected maize tissue for 2 days, then transferring them to healthy maize for 14 days. The leafhoppers were then starved for 1 h and allowed to inoculate healthy plants for 0.5, 1, 5, 10, 15, 20, and 24 h.

To determine the minimum time for transmission, and thus, indirectly, the minimum latent period after acquisition before the leafhopper could transmit virus, *G. sonora* leafhoppers were allowed a 48-h

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acquisition period, then transferred to healthy plants for 7, 9, 11, or 14 days at 30°C. Tests of temperature optimum were done by allowing *G. sonora* leafhoppers a 48-h acquisition period at 25°C followed by a 14-day inoculation period at 16, 18, 24, 30, or 36°C. The plants were monitored for symptoms, and selected plants were assayed for virus by ELISA.

To test for ability to transmit SSMV, *G. nigrifrons* and *P. maidis* were given a 48-h acquisition period followed by a 14-day inoculation period at 27°C. Plants were monitored for symptoms. Ten adult leafhoppers per plant and 15 plants were used for each leafhopper species. To compare the ability of female and male *G. sonora* to transmit SSMV, after acquisition, five leafhoppers of each sex were allowed to feed on each of 15 plants for a 14-day inoculation period.

The host range of SSMV was tested by allowing leafhoppers a 48-h acquisition

access period on infected plants followed by a 20-day feeding on healthy plants of each of the following species of sorghum relatives: *Sorghum halepense* (L.) Pers. (johnsongrass), *S. bicolor* (L.) Moench (wild cane), and *S. sudanense* (Piper) Stapf. cvs. Double Dwarf and Piper (Sudan grass). Known SSMV hosts *Triticum aestivum* L. cv. Yecoro Rojo, *Avena sativa* cv. Cal Red Oats, and *Z. mays* cv. Golden Bantam were tested for comparison (10). Five leafhoppers were fed on 10 plants of each species at the four- to five-leaf stage, and the experiment was done twice.

ELISA detection of SSMV. An indirect ELISA was used to detect SSMV in plants, as in Creamer (5). Corn samples were ground with a sap expressor (Piedmont Tool and Die, Seneca, SC) and diluted 1:4 (wt/vol) in coating buffer. Samples (200 µl per well) were added to Immulon 2 microtiter plates (Dynatech, Chantilly, VA) and incubated overnight at 4°C. Anti-

SSMV IgG was used at 1.6 µg/ml, alkaline phosphatase-conjugated goat anti-rabbit IgG was used at 1:600 dilution, and incubations were done for 2 h at 37°C. *p*-Nitrophenyl phosphate was added at 0.6 mg/ml for color development. Results were assessed spectrophotometrically at 405 nm with an E Max microplate reader (Molecular Devices, Palo Alto, CA). Plants were considered infected if the absorbance was greater than three times the average of the healthy plant controls.

Double antibody sandwich (DAS)-ELISA (3) was used to detect SSMV in leafhoppers. Leafhoppers were ground in microfuge tubes in 100 µl of sample buffer. Anti-SSMV IgG was used at 1.6 µg/ml to coat plates, and alkaline phosphatase-conjugated anti-SSMV IgG was used at 1:600 dilution. Incubations were done for 3.5 h at 37°C, with color development and results assessed as above. Leafhoppers were considered infected if the absorbance was greater than the mean of 15 individual healthy leafhoppers plus 3 SD.

RESULTS

G. sonora transmitted SSMV after a minimum AAP of 6 h with 13.3% average efficiency, while 100% transmission was not achieved until after a 48-h AAP (Table 1). Following a 48-h AAP and a 14-day incubation period, *G. sonora* was capable of inoculating corn during a minimum feeding period of 1 h (13.3% average efficiency), and leafhoppers given a 24-h IAP transmitted to an average of 83.3% of the plants (Table 2). A shorter IAP of 0.5 h gave no transmission.

Transmission efficiency was highest (96.7%, 29/30) at temperatures of 30°C or 36°C, while transmissions carried out at 24°C were slightly less efficient (83.3%, 25/30). No transmission (0 out of 39 plants) occurred at 16°C, while low levels of transmission (35%, 21/60) were found at 18°C. After a 48-h AAP, *G. sonora* transmitted SSMV after a minimum of 9 days of feeding on healthy corn at 30°C; a 14-day transmission feeding gave 100% transmission (Table 3).

To determine if the lack of transmission at lower temperatures was due to poor virus replication, plants were held at 16, 20, or 30°C for 14 or 28 days after acquisition. At the conclusion of the incubation periods, the leafhoppers were removed and tested singly by ELISA, and the plants were rated for symptoms. No insects (0/25) and no plants (0/12) showed SSMV infection when the 14-day incubation was done at 16°C, while 17% (4/24) of the leafhoppers and 67% (8/12) of the plants were infected with virus when the temperature was increased to 20°C. After the 14-day incubation at 30°C, 52% (13/25) of the *G. sonora* and 100% (12/12) of the plants contained SSMV. Increasing the incubation period to 28 days increased virus infection in the leafhoppers (53%, 8/15) and in the

Table 1. Transmission rate of sorghum stunt mosaic rhabdovirus (SSMV) by *Graminella sonora* with varying acquisition access periods (AAP)

AAP ^x (h)	Percent infection ^y			Mean transmission rate ^z
	Trial 1	Trial 2	Trial 3	
3	0	0	0	0 a
6	10	10	20	13.3 b
12	20	10	20	16.7 b
24	70	80	90	80.0 c
48	100	100	100	100.0 d

^x Leafhoppers were fed on healthy maize test plants for 14 days postacquisition, and plants were assayed for virus 21 days postinoculation.

^y Ten test plants per time period were inoculated in each trial with five leafhoppers per plant.

^z Numbers followed by the same letter are not significantly different ($P < 0.05$) using Fisher's PLSD.

Table 2. Transmission rate of sorghum stunt mosaic rhabdovirus (SSMV) by *Graminella sonora* with varying inoculation access periods (IAP)

IAP ^w (h)	Percent infection ^x			Mean transmission rate ^y
	Trial 1	Trial 2	Trial 3	
0.5	0	NT ^z	0	0 a
1	10	20	10	13.3 b
5	20	NT	20	20.0 bc
10	30	20	20	23.3 bc
20	30	30	40	33.3 c
24	100	80	70	83.3 d

^w Leafhoppers were fed on SSMV-infected maize for 48 h, then on healthy plants for 14 days prior to the inoculation feeding.

^x Ten test plants per time period were inoculated in each trial with five leafhoppers per plant.

^y Numbers followed by the same letter are not significantly different ($P < 0.05$) using Fisher's PLSD.

^z Not tested.

Table 3. Transmission rate of sorghum stunt mosaic rhabdovirus (SSMV) by *Graminella sonora* with varying incubation periods

Incubation period ^x (days)	Percent infection ^y			Mean transmission rate ^z
	Trial 1	Trial 2	Trial 3	
7	0	0	0	0.0 a
9	60	70	50	60.0 b
11	100	100	80	93.3 c
14	100	100	100	100.0 c

^x Leafhoppers were fed on SSMV-infected maize for 2 days. Incubation period = total days fed on healthy plant.

^y Ten test plants per time period were inoculated in each trial with five leafhoppers per plant.

^z Numbers followed by the same letter are not significantly different ($P < 0.05$) using Fisher's PLSD.

maize (91%, 10/11) held at 16°C up to the level of virus infection of leafhoppers (47%, 7/15) and plants (100%, 8/8) held at 30°C.

No differences in transmission were found between male (67%) and female (60%) *G. sonora*. SSMV was not transmitted by *G. sonora* to wild cane, johnsongrass, oats, or either variety of Sudan, while all wheat and maize plants were infected (100%). *G. nigrifrons* and *P. maidis* were not found to transmit SSMV.

DISCUSSION

The transmission parameters determined for SSMV are generally similar to those found for the other hopper-transmitted rhabdoviruses infecting monocots. The minimum AAP of 6 h found for SSMV is similar to the 5 h reported for BYSMV (4), but substantially longer than the 5-min minimum acquisition time reported for RYTV (2) and 30-sec AAP reported for WSMV (12). The minimum IAP for SSMV of 1 h is longer than the 15-min minimum IAP reported for WSMV (12); however, WSMV was also transmitted very efficiently (>80%) after 24 h, which is similar to what we found with SSMV. The minimum latent period of 9 days for SSMV is very similar to that of RYTV (2) and MMV (1), and is much longer than the 4 days reported for WSMV (12).

The high level of SSMV transmission at 24 to 36°C is similar to that of RYTV (8). The lack of SSMV transmission at 16°C was not due to poor leafhopper survival, since most of the leafhoppers could be recovered after the 14-day IAP. The efficient virus transmission at 16°C after doubling the incubation period suggests that the incubation period of the virus increased

at lower temperatures. Similar results were found with RYTV (8), which was not transmitted at 16°C and required an average of 23 days incubation period prior to transmission at 20°C. Our findings, that increasing incubation temperatures increased the number of leafhoppers infected with SSMV, supports the hypothesis that viral replication rate increases with increasing temperature.

It was surprising to us that *G. nigrifrons* did not transmit SSMV because of its close relationship with *G. sonora* (11). This *Graminella* species is also a grass generalist and can be reared in the laboratory on the same hosts as *G. sonora*. In addition, both leafhoppers transmit other viruses, such as maize chlorotic dwarf virus, and give similar waveform patterns using an electronic insect feeding monitor (14). Interestingly, the transmission parameters found for *G. sonora* transmission of SSMV were most similar to those of other rhabdoviruses transmitted by both leafhoppers and plant hoppers. Thus, no correlation was found between the type and relatedness of the vector and the transmission parameters.

The SSMV host range we found was limited to corn, wheat, and sorghum, as previously reported by Mayhew and Flock (10). This host range is more similar to MMV than to any other monocot-infecting rhabdovirus (6). However, the alternate hosts of SSMV are still unknown. With the seasonal and sporadic growth of corn or sorghum in the Imperial Valley of California, alternate hosts are likely required for the seasonal survival of SSMV.

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