

**TRANSMISSION OF *DIGITARIA STREAK VIRUS* BY THE  
*MAIZE STREAK VIRUS* LEAFHOPPER VECTOR  
*CICADULINA MBILA* NAUDÉ**

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**Running Title: *Trans-encapsidation of DSV***

**Figures: 2**

## SUMMARY

We investigated the potential for transmission of Digitaria streak virus (DSV) by the vector leafhopper of the related but distinct mastrevirus Maize streak virus (MSV), from mixed infections of the two viruses. A dimerised clone of the genome of DSV was cloned in an *Agrobacterium tumefaciens* vector plasmid and shown to be agroinfectious in sweetcorn. An infectious 1.1-mer of an MSV genome was cloned in tandem with the agroinfectious DSV construct, so that sweetcorn seedlings could be simultaneously agroinfected with both DSV and MSV-Kom. DSV could be transmitted by the MSV leafhopper vector *Cicadulina mbila* Naudé from these doubly infected plants to uninfected sweetcorn, but not from singly-infected plants, although DSV DNA was detected by PCR in both sets of leafhoppers. This could either indicate that MSV-Kom coat protein can *trans*-encapsidate the DSV genome, or that particle aggregations of both viruses are transmissible. Possible implications of this finding for epidemiology of mastreviruses are discussed.

*Maize streak virus* (MSV) and *Digitaria streak virus* (DSV) are related but distinct species of the genus *Mastrevirus* (family: *Geminiviridae*). These viruses all have monopartite circular ssDNA genomes, geminate particles, and are obligately transmitted by different genera of leafhoppers [5];[15]. Unlike the two-component whitefly-transmitted begomoviruses, mastreviruses require essentially the whole genome for infectivity and movement in plants, including the coat protein (CP) gene ([14]; [4]; see also [15]). It is not clear whether the requirement for the CP represents a requirement for encapsidation, or whether the coat protein functions in movement of nucleoprotein complexes.

Briddon *et al.* [6] showed that the viral coat protein is the sole determinant of insect vector specificity for geminivirus transmission, by replacing the coat protein gene of the whitefly-transmitted African cassava mosaic virus (ACMV; genus: *Begomovirus*) with that of the leafhopper transmitted Beet curly top curtovirus (BCTV). Recombinant viruses were infectious, and could be transmitted by leafhoppers after intrahaemocoelic injection. This showed that both the DNA A and DNA B components of the chimaeric ACMV were encapsidated by the BCTV coat protein, and implies that there is no specific encapsidation signal present on the virus genome.

We addressed the question of whether non-vector leafhopper species could transmit a mastrevirus from a mixed infection by co-agroinfecting sweetcorn seedlings with MSV and DSV on the same *Agrobacterium tumefaciens* plasmid, and subsequently attempting leafhopper transmissions using the MSV vector *Cicadulina mbila* Naudé. MSV is transmitted by *Cicadulina* spp. [19], while DSV is transmitted by *Nesoclutha declivata* Linnavuori and cannot be transmitted by *C. mbila* [12]; [18]. While not specifically addressing the question of *trans*-encapsidation of DSV by the MSV coat protein, it was of interest in epidemiological terms to determine whether mixed infections of mastreviruses would allow non-vector transmission.

All standard molecular biological techniques were performed as described in [21], and according to the specifications of enzyme manufacturers.

MSV and DSV were routinely cloned as tandem dimers for introduction into *Agrobacterium* binary vectors pBI121 (Clontech, Palo Alto, CA.) or pBin19 [2].

An agroinfectious genomic clone of MSV (MSV-Kom, Genbank AF003952) was supplied by F.L. Hughes and W.H. Schnippenkoetter (this laboratory). Plasmid pKom500 contains the genome in pUC19; pKom602 contains a 1.1-mer (tandem clone of long intergenic region plus whole genome) of the MSV-Kom genome in pUC19; pKom603 contains a 1.1-mer of the MSV-Kom genome in pBI121.

The full genome of DSV was previously cloned in pUC18 (pDSV100; [11]). We cloned a tandem dimer of this genome to yield pDSV200. The viral insert from this was cloned in the *Hind*III site of pBin19, generating pDSV200Bin.

We constructed a plasmid containing infectious genomes of both DSV and MSV cloned on the same T-DNA. The pUC18-based pDSV200 and pKom602 were digested with *Xmn*I and partially with *Hind*III and sticky ends blunted with the Klenow fragment of DNA polymerase I. The appropriate linear fragments were gel purified and ligated to create pDSV-MSV, with the insert flanked by *Eco*RI sites. The 8.4-kb *Eco*RI fragment from pDSV-MSV was cloned into the *Eco*RI site of pBin19. The recombinant plasmid with the DSV dimer proximal to the right border of the T-DNA was named pDSV-MSVBin: this would ensure that the DSV dimer would enter cells before the MSV 1.1-mer [24].

*Agrobacterium tumefaciens* C58C1(pMP90) [13] was used for agroinfections. Transformation was by the freeze-thaw method of Holsters *et al.* [9]. Transformants were selected and maintained on LA plates containing rifampicin (100 µg/ml), gentamycin (40 µg/ml) and kanamycin (100 µg/ml).

For agroinoculations *A. tumefaciens* was grown overnight in LB media with antibiotic selection, concentrated by centrifugation, and resuspended in 1/5 volume of sterile distilled water. Maize kernels (*Zea mays* cv. “Jubilee”) were germinated at 30° C in damp sterile vermiculite and three day-old maize seedlings agroinoculated

according to [8]. Injected seedlings were grown at about 25° C with a 16: 8 hour day/night cycle. Infections were scored from 5 days post-inoculation.

We used the method of [16] for isolation of plant DNA. DNA was isolated from individual leafhoppers by the method of [23].

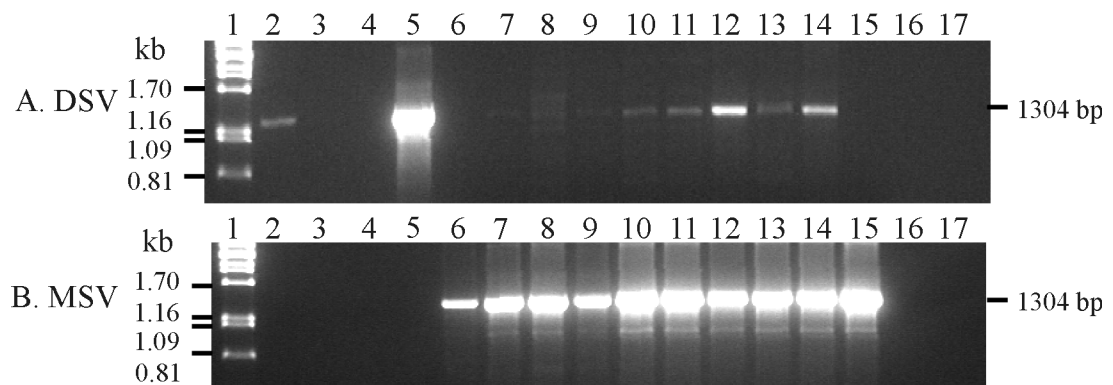
Detection of viral sequences in plants and insects was by polymerase chain reaction (PCR) amplification. About 10 ng of total plant DNA, or 1 µl of a total of 100 µl of nucleic acid extracts from individual leaves, was used in each PCR. For detection of MSV-Kom sequences primer sequences were derived from the MSV-Kom sequence positions 1770-1792, and 215-234 [17]. The PCR cycle conditions were: 95° C / 120 sec; 30 x (94° C / 45 sec, 54° C / 45 sec, 72° C / 90 sec), and 72° C for 180 sec. For detection of DSV we used primer MSV1770-1792 (which anneals to the DSV genome), and primer DSVrev (5'-CTCGGCGGGACCAAATTCAA-3') which anneals to DSV DNA in the MP gene / V1 ORF. The PCR cycle conditions were: 95° C / 120 sec; 30 x (94° C / 45 sec, 54 °C / 30 sec, 72° C / 75 sec), and 72° C for 5 min.

Leafhoppers (*C. mbila* Naudé) were maintained as described elsewhere [7]. Non-viruliferous leafhoppers were transferred on maize leaf material from the stock colonies into gauze-covered hurricane lamp glasses. The leafhoppers were then placed on infected plant material in larger cages and allowed to acquire virus by feeding on the infected material for two to three days. After the 2 - 3 days acquisition period, uninfected sweetcorn cv. Jubilee seedlings (7 - 10 days old) were placed into the cages and infected plants cut down. Viruliferous leafhoppers were allowed to feed on the uninfected sweetcorn for five to seven days, which was then sprayed and transferred to a plant growth room.

Three day old seedlings of the sweetcorn cv. Jubilee were agroinfected with pDSV200Bin and pDSV-MSVBin. Out of 25 seedlings which survived injection with pDSV200Bin, 10 (40%) showed definite symptoms. Agroinfection with pDSV-MSVBin resulted in 17 out of 21 seedlings (81%) exhibiting symptoms. Symptoms caused by DSV were extremely mild on sweetcorn, consisting of very

few and widely dispersed streaks, in contrast with those typically associated with MSV-Kom infection (not shown; see <http://www.mcb.uct.ac.za/MSV/msvsymptom.htm>).

We agroinfected plants with both DSV and MSV, and used *C. mbila* to transmit virus from one of these plants to sweetcorn seedlings. The results of PCR amplification of viral DNA from leafhoppers and plants is shown in Figure 2. Detection of DSV DNA in one of three *C. mbila* which had been feeding on DSV-infected plant material (lane 2) and from two or three of the four leafhoppers (lanes 7, 8 & 9) which had been feeding on the doubly infected plant, showed that *C. mbila* can take up DSV DNA - presumably in the form of virions - but not that they can transmit the virus. However, results in Figure 1 show that DSV was transmitted from a plant doubly infected with MSV and DSV (lane 14) to each of four individual plants (lanes 10 to 13).



**Figure 1: Detection of DSV and MSV-Kom sequences in plants and in *C. mbila***

The same DNA extracts were amplified in separate PCRs. Gel A shows the results of PCR with primers MSV 1770-1792 and DSVrev (specific for DSV). Gel B shows the results of PCR with primers MSV 1770-1792 and MSV 213-234 (specific for MSV). Lane 1: molecular weight marker ( $\lambda$  DNA digested with *Pst*I); lanes 2, 3 & 4: DNA amplified from *C. mbila* individuals which had been feeding on plant A agroinfected with pDSV200BI; lane 5: acquisition plant A - infected with DSV by agroinfection with pDSV200Bin; lanes 6 to 9: DNA amplified from *C. mbila* individuals fed on plant B agroinfected with pDSV-MSVBin; lanes 10 to 13: DNA amplified from the first infected leaves of plants which had been infected by leafhopper transmission from plant B.; lane 14: DNA amplified from acquisition plant B; lane 15: DNA amplified from a plant agroinfected with pKom603; lane 17: negative control - PCR amplification from a plant exposed to *C. mbila* which had been feeding on plant A.

Variation in the intensity of amplification probably reflects differing concentrations of DSV in plants. Sweetcorn is not a good host for DSV, and the DSV DNA could only be detected by PCR (not shown). In a similar experiment, Boulton [3] found no transmission of DSV by *C. mbila* in the presence of MSV-N by Southern blot assay;

however, in their case two cultures of *Agrobacterium tumefaciens* were mixed, and it is not certain that both viral genomes were present in the same cells. In our experiments, cloning of partial multimers of both virus genomes on a single plasmid ensures simultaneous transfer into the same recipient cell by *Agrobacterium tumefaciens*. More dramatic data may have been obtained if the DSV vector *Nesoclutha declivata* had been available to transmit MSV to a susceptible host such as maize or *Digitaria*; however, the vector was not available.

The transmission of DSV from doubly-infected plants by *C mbila*, and the lack of success in transmission from singly-infected plants, could imply that the MSV-Kom coat protein encapsidated the DSV genome in double infections. It is certain that the viral coat protein, and therefore encapsidation of the viral genome, is required for insect transmission [1]: no other mechanism of uptake of the viral genome by leafhoppers or other insect vectors has been described. However, it could also imply that mixed aggregates of homogeneously-assembled virions could be taken up by leafhoppers, given that this is apparently the means of uptake of MSV [18]. The possibility could be investigated by swapping coat protein genes between the viruses, much as was done by Briddon *et al.* [6] for ACMV and BCTV; however, this was not addressed in this investigation.

If the transmission of mixed populations of mastreviruses by a single vector of one of them does occur in nature, the process may play an important role in the maintenance of mixed populations of viruses in reservoir hosts. In this case, it would potentially allow the survival and onward transmission of DSV in a background of MSV, in the absence of its own vector. In natural circumstances, such as in African grasslands, it might allow maintenance of viruses in a variety of host species, despite widely differing efficiencies of transmission by the preferential vector species to particular hosts: there is evidence that natural infections of MSV, for example, contain a wide variety of viral genotypes, albeit some at very low concentration ([10]; DP Martin and EP Rybicki, unpublished). Although *C mbila* is the predominant vector of maize isolates of MSV, other *Cicadulina* species can also transmit MSVs and other African streak viruses [22]; [19]: it is possible that survival

of different viruses in natural conditions is improved by mixed infections, if not by actual *trans*-encapsidation. The phenomenon may explain some hitherto unconsidered aspects of geminivirus epidemiology, especially where viruses may be seen to be transmitted in the apparent absence of a suitable vector.

This sort of transmission may also play an important rôle in the evolution of new geminivirus species, as it may help the virus to expand its host range to species which are not normally favoured by their primary insect vector. Recombination is likely to play an important role in evolution of geminiviruses; for example, between leafhopper-transmitted mastre- or curtoviruses and whitefly transmitted begomoviruses. In fact, curtoviruses are probably the result of recombination between a whitefly-transmitted geminivirus and a leafhopper-transmitted virus (see [20]). This event would have to have taken place in plants where there were mixed infections, and it is possible that *trans*-encapsidation of viral genomes played a role in the evolution process. This would have ensured the persistence of the mixed infection until a viable recombinant evolved, since both whiteflies and leafhoppers might have been able to transmit both virus species, albeit at low efficiency.

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