

**Soybean blotchy mosaic virus:
Molecular characterization and seasonal
persistence**

by

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Declaration

I, Elrea Strydom, hereby declare that this thesis submitted to the University of Pretoria for the degree PhD Microbiology contains my own work, and that the content contained within this thesis has not been submitted to any other university or institution.

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Abstract

Infection of soybean by the plant cytorhabdovirus Soybean blotchy mosaic virus (SbBMV) leads to the development of characteristic blotchy mosaic symptoms on leaves. These symptoms re-appear early in each new soybean production season and result in significant yield losses. The persistence of SbBMV between seasons may occur through the presence of reservoir plant hosts during winter, seed transmission or propagation in the leafhopper vector of SbBMV, *Peragallia caboverdensis* as reported for other plant rhabdoviruses. The first aim of this study was to identify alternative hosts of SbBMV in the areas surrounding soybean fields. SbBMV was successfully detected in *Gymnosporia buxifolia*, *Flaveria bidentis* and *Lamium amplexicaule*, and these plant species may serve as viral reservoirs in the absence of soybean. This was followed by a seed transmissibility trial on four commercial soybean cultivars, in which transmission of SbBMV through seed could not be detected. The absence of seed transmissibility and the low frequency of SbBMV mechanical transmission implies that transmission by an insect vector, possibly *P. caboverdensis* is the main method by which SbBMV is spread. The role of *P. caboverdensis* in the dissemination of SbBMV was thus investigated. The year-round presence of *P. caboverdensis* was confirmed at the study site and its association with SbBMV confirmed. *P. caboverdensis* thus has the potential to spread SbBMV between soybean and alternate hosts year-round. A positive strand-specific RT-PCR (pss-RT-PCR) capable of detecting replication of SbBMV was developed to examine the mode of SbBMV transmission by *P. caboverdensis*. No replicative forms of SbBMV, indicative of persistent, propagative transmission of SbBMV, were obtained in any of the leafhopper individuals tested. The first DNA sequence data for *P. caboverdensis* was generated with the sequencing of regions of the mitochondrial cytochrome c oxidase subunit I (*mtCOI*) and histone 3 (*H3*) genes, which will aid species identification without a requirement for morphological expertise. The amount of genetic diversity present in SbBMV populations from different geographical locations, hosts and time of collection was also investigated. Three main lineages, mostly corresponding to geographic origin, were identified. In future, the survey for identification of alternate hosts

should be expanded in order to determine whether additional plant reservoir hosts exists. The establishment of a *P. caboverdensis* colony will allow for the collection of conclusive evidence for the mode of transmission of SbBMV by *P. caboverdensis* by monitoring replication and viral load in insects using the pss-RT-PCR and RT-qPCR respectively. Partial *mtCOI* and *H3* gene sequences should also be determined for additional *P. caboverdensis* accessions from different geographical origins, as well as closely related leafhopper species to confirm accurate species delineation using these two markers. Finally, determination of the whole genome sequence of SbBMV isolates will allow for a phylogenomic approach to determining the evolutionary relationships and genetic diversity present in SbBMV populations. Implementation of chemical control measures such as systemic insecticides, and improved farming and management practices aimed at removing alternate hosts may limit production losses as a result of SbBMV.