

# Disease Note

## Diseases Caused by Viruses

### First Report of *Soybean mosaic virus* in Commercially Grown Soybean in the Netherlands

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In July 2020, plants with crinkled, chlorotic, occasionally necrotic leaves, typical for soybean mosaic virus (SMV), were observed in eight soybean fields (*Glycine max* L.) in Flevoland, the Netherlands. Disease incidence varied from 5 to 50%, and the plants affected often occurred in small or extensive patches. Leaves from several symptomatic plants were sampled from each of two fields planted with soybean variety Green Shell or Summer Shell. Total RNA was extracted from one plant leaf sample per field using InviTrap Spin Plant RNA Mini Kit (Invitex, Germany). One-tube RT-PCRs employing potyvirus generic primers P9502 and CPUP (Van der Vlugt et al. 1999) and SMV-specific primers SMV-dT (5'-TTTTTTTTTTTTTTAGGACAAC-3') and SMV-Nib-Fw (5'-CAAGGATGARTTTAAGGAG-3') combined with Sanger sequencing confirmed the presence of SMV in all leaf samples. To exclude the presence of other agents in the samples, total RNA from each cultivar was used in standard Illumina library preparation with ribosomal RNA depletion followed by sequencing on an Illumina NovaSeq6000 (paired-end, 150 bp) which yielded 66,579,158 reads (Summer Shell) and 223,953,206 reads (Green Shell). After quality trimming in CLC Genomics Workbench 20.0.4 (CLC-GWB; Qiagen, Germany), four million reads were randomly sampled for de novo assembly. Contigs over 500 nucleotides (nt) in length with a minimum of 500 reads were annotated by BLASTn against NCBI GenBank. This identified one contig of 9,883 nt (6,233,397 reads) in Summer Shell and one contig of 9,727 nt (3,139,927 reads) in Green Shell with clear homology to SMV (E-value = 0.0). No other viruses were identified in the datasets. Reference assemblies against the SMV reference

sequence (NC\_002634) mapped 24,090,763 reads (36.2%) for Summer Shell and 175,459,637 reads (78.3%) for Green Shell. Extracted consensus sequences for SMV in both soybean cultivars were 9,584 nt long (excluding the poly-A tail). Sequence data from the de novo and reference assemblies were combined into consensus sequences that showed over 98% overall nt sequence identity to NC\_002634 and 99.6% to each other. Both consensus sequences were deposited in GenBank under accession numbers MW822167 (SMV-Summer Shell) and MW822168 (SMV-Green Shell). In addition, the presence of SMV in the field samples was confirmed with an inoculation assay. Leaf samples from both fields were ground in phosphate buffer (0.1M, pH 7.2) and inoculated on cotyledons and first expanded leaves of soybean plants (unknown cultivar) 12 days postgermination. Plants showed veinal chlorosis in systemic leaves from 12 days postinoculation, which developed into veinal necrosis. SMV infections were confirmed by RT-PCR in systemic, chlorotic leaf samples of all symptomatic plants using the SMV-specific primers described above. To our knowledge, this is the first report of SMV in the Netherlands. As soybean is a relatively new but expanding crop in this country, information about emerging diseases is highly relevant. SMV can be transmitted via seeds and aphids, where seeds can serve as primary source of virus inoculum (Cui et al. 2011; Hajimorad et al. 2018; Hartman et al. 2016). Weeds and noncommercial plants can also serve as origin of SMV, particularly in subsequent growing seasons, although this virus infects a limited host range of six plant families (Cui et al. 2011; Hill and Whitham 2014). Special monitoring would be advised for the recurrence and possible damage by SMV in Dutch soybean fields.

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