Identification of a *Cucumber mosaic virus* Subgroup II Strain Associated with Virus-like Symptoms on *Hosta* in Ohio

**John R. Fisher,** Ohio Department of Agriculture, Plant Health Diagnostic Laboratory, Plant Health Division, Reynoldsburg, OH 43068

Corresponding author: John R. Fisher. jfisher@agri.ohio.gov


*Cucumber mosaic virus* (CMV) is the type species of the *Cucumovirus* genus in the Family *Bromoviridae*. The virus is distributed worldwide, has a very broad host range infecting over 1000 species in more than 85 plant families, and is transmitted by more than 80 aphid species in 30 genera in a non-persistent fashion. The viral genome is positive sense, single-stranded RNA divided among three segments which encode five proteins. RNAs 1 and 2 encode 3 proteins with methyl transferase/helicase, replicase, and suppression of RNA silencing functions. The movement protein (MP) gene is expressed directly from the 5’ half of RNA 3, and the coat protein (CP) gene is expressed from the 3’ half via a subgenomic RNA, referred to as RNA 4. CMV is divided into two subgroups based on serological and nucleotide sequence relatedness. Some isolates are also reported to harbor small satellite (sat) RNAs (3).

In the spring of 2012, a *Hosta* sp. 'Cynthia' sample displaying virus-like symptoms, including mottle and spotting (Fig. 1), was submitted to the Ohio Plant Diagnostic Network as part of a Farm Bill funded survey of perennial viruses. The sample tested positive for CMV and negative for the *Potyvirus* group, *Alfalfa mosaic*, *Arabis mosaic*, *Impatiens necrotic spot*, *Tobacco mosaic*, *Tobacco ringspot*, *Tobacco streak*, *Tomato mosaic*, *Tomato ringspot*, and *Tomato spotted wilt* viruses by ELISA using commercially available antibodies (Agdia Inc., Elkhart, IN). The sample also tested negative for *Tobacco rattle virus* by reverse transcription (RT) PCR.
Fig. 1. Mottle and spotting symptoms observed on Hosta sp. ‘Cynthia’ plants.

Double-stranded (ds) RNA was purified from leaf tissue as previously described (4), resulting in a dsRNA banding profile atypical for CMV (Fig. 2). cDNAs were synthesized from a dsRNA template and used to amplify the MP and CP regions of the genome by PCR as previously described (1,2). The cDNAs were also used in PCR with primers designed to amplify satRNA (1). The MP and CP primers amplified strong, clear products of the expected size, approximately 1200 and 1000 bp, respectively (Fig. 3). The amplicons were excised from the gel, purified, and ligated into pGEM-T vector as previously described (1,2). Colonies were screened for an insert by PCR using M13 primers, and the plasmid DNA was purified and subsequently sequenced (Plant Microbe Genomics Facility, The Ohio State University). Vector sequences were trimmed from raw sequences (Chromas v. 2.33), assembled, and subjected to pairwise and multiple sequence alignments (Vector NTI Advance 11.5, Invitrogen) as previously described (1,2).
Fig. 2. dsRNA profiles obtained from (Lane 1) Hosta sp. 'Cynthia' leaf tissue and (Lane 2) CMV-Vinca N1-03 isolate from tobacco tissue. CMV dsRNAs 1-4 and satellite dsRNA are indicated to the right. M = 1 Kb DNA ladder (250-4000 bp markers indicated to the left). Electrophoresis was done in 1% agarose at 100 volts for 90 min in 1X TAE buffer. Gel was stained with ethidium bromide.
Three MP and four CP amplicon clones were sequenced and the processed sequences deposited in GenBank (accession nos. JX898514-JX898520). All of the MP clones were 1196 nucleotides (nt), with the MP open reading frame (ORF) being 840 nt. All of the CP clones were 973 nt, with the CP ORF being 657 nt. BLASTn searches of the NCBI database using the MP and CP ORF sequences produced matches with 99% nt identities; notably the CMV subgroup II strain LS (accession no. AF127976.1) and a subgroup II isolate we recently reported from *Vinca minor* (accession nos. JF918960.1, JF918965.1) (1). These results represent the first confirmed report of CMV infecting *Hosta* spp. in Ohio.

The CMV-*Hosta* isolate described here is clearly a subgroup II strain closely related to strain LS, as well as one found in *Vinca minor* in Ohio. Further, the CMV-*Hosta* isolate did not have detectable satRNA associated with it based on dsRNA and PCR results. The dsRNA profile obtained from the CMV-*Hosta* isolate was atypical in that none of the genomic dsRNAs were visible when subjected to agarose gel electrophoresis (Fig. 2), yet cDNAs synthesized from purified dsRNA template amplified strong products with the MP and CP markers demonstrating the presence of RNA 3 in the sample extract and suggesting the genomic dsRNA titre was too low to detect visually. These results expand the known host range for CMV in Ohio to include *Hosta* spp. and benefit *Hosta* growers by increasing awareness of the virus as a current threat, especially since the symptoms initially observed on *Hosta* ‘Cynthia’ disappeared over the course of the growing season.
**Literature Cited**


