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First Report of *Tobacco ringspot virus* Infecting *Ajuga reptans* in Ohio

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Tobacco ringspot virus (TRSV) is the type species of the *Nepovirus* genus of the Family *Secoviridae*. The virus has a bipartite, single-stranded positive sense RNA genome that is translated into two polyproteins. The RNA 1 polyprotein has six domains and includes the RNA dependent RNA polymerase at its 3' terminal. The RNA 2 polyprotein has three domains and includes the coat protein at its 3' terminal. TRSV has a broad host range and is transmitted by longidorid nematodes (3).

In the fall of 2011, a bugleweed (*Ajuga reptans* 'Bronze Beauty') sample exhibiting a very subtle virus-like mottle symptom on new growth (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 TRSV and negative for the *Potyvirus* group, *Alfalfa mosaic virus* (AMV), *Arabidopsis mosaic virus*, *Cucumber mosaic virus* (CMV), *Impatiens necrotic spot virus*, *Tobacco mosaic virus*, *Tobacco streak virus* (TSV), *Tomato mosaic virus*, *Tomato ring spot virus*, and *Tomato spotted wilt virus* by ELISA using commercially produced antibodies (Agdia Inc., Elkhart, IN). The sample also tested negative for *Tobacco rattle virus* by RT-PCR. Previously AMV, CMV, and TSV have been reported in bugleweed in Ohio (1) but this is the first confirmed report of TRSV infecting *A. reptans* in the state.

Fig. 1. Subtle virus-like mottle symptom observed on new growth of *A. reptans* 'Bronze Beauty' plant infected with TRSV.

For immunocapture reverse transcription (IC-RT), magnetic beads conjugated with sheep anti-rabbit IgG were incubated with polyclonal rabbit anti-TRSV IgG (Agdia Inc., Elkhart, IN) as previously described (2). Leaf tissue samples were extracted, incubated with TRSV-IgG conjugated beads, washed, and cDNAs synthesized from bound virions (2). Two full length and two partial TRSV RNA 1 sequences (accession #: U50869.1, NC005097.1, AJ698718.1, EF528581.1), and two full length and three partial TRSV RNA 2 sequences (accession #: AY363727.1, NC005096.1, HQ446459.1, HQ446463.1, HQ446464.1) were used to design two sets of primers to amplify the RNA

dependent RNA polymerase (RdRp) region of RNA 1 (TRSVRdRpfd6005: 5'-GCTGATTGCGACCCAATACCTC-3'; TRSVRdRpfd7067: 5'-GCTACCAATCCTGGTTAGAAG-3') and the coat protein (CP) region of RNA 2 (TRSVCPfd2442: 5'-GGTGACACTTTCGCCATTG-3'; TRSVCPfd3452: 5'-GGACAAAACACCTAACTAAAG-3'; Integrated DNA Technologies Inc., Coralville, IA). Five μ l of cDNA or sterile water was used as template for individual PCR reactions. Amplification was done in 25 μ l reactions (1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 μ M primer pair, 0.625 units GoTaq Flexi polymerase (Promega Inc., Madison, WI) using the cycling parameters: 94°C (2 min), 30 cycles of 94°C (45 sec), 51°C (30 sec), 47°C (30 sec), 72°C (60 sec), and a final extension of 72°C (10 min).

cDNAs synthesized from immunocaptured virions amplified strong, distinct products of expected size with the RdRp and CP primers (Fig. 2). The amplicons were excised, purified from the agarose, and cloned into pGEM-T vector as previously described (2). Colonies were screened for inserts which were subsequently sequenced. Sequences were assembled and subjected to pairwise and multiple sequence alignments (2). Eight RdRp and six CP amplicon clones were sequenced and the processed sequences deposited in GenBank (accession numbers: JX848602-JX848615). All of the RdRp clones were 1055 nucleotides (nt) and all of the CP clones were 1013 nt. RdRp clones shared 99.4-100% nt identity with a mean identity of 99.7%, with CP clones sharing 99.7-99.9% nt identity with a mean of 99.8%. A BLASTn search of the NCBI database using default algorithm settings identified a single sequence (accession # U50869.1) with 89% nt identity (100% query coverage) to the RdRp clones, and a single sequence (accession # AY363727.1) with 97% nt identity (100% query coverage) to the CP clones. When translated, the RdRp clones corresponded to amino acids (aa) 1969-2301 of the RNA polymerase region of the RNA 1 polyprotein and shared 96.1% predicted aa identity with accession # U50869.1. The CP clones corresponded to aa 774-1101 of the coat protein region of the RNA 2 polyprotein and shared 99.1% predicted aa identity with accession # AY363727.1. These results represent the first confirmed report of TRSV infecting bugleweed in Ohio and expand the known host range of the virus for the state. Growers should also benefit from these results by gaining awareness of TRSV as a potential threat to their operations, and of bugleweed as a perennial reservoir for the virus.

Fig. 2. PCR detection of TRSV from cDNAs synthesized from immunocaptured virions with RdRp-specific primers (Lane 1) and CP-specific primers (Lane 2). Water controls with RdRp primers (Lane 3), CP primers (Lane 4), and M13 plasmid primers (Lane 5). TSV CP clone used as a positive PCR control with M13 primers (Lane 6). M=1 Kb DNA ladder (250, 500, 750, 1000, and 1500 bp markers indicated). Electrophoresis was done in 0.8% agarose at 100 volts for 60 minutes in 1X TAE buffer. Gel was stained with ethidium bromide.

Literature Cited

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