Molecular Detection of *Tobacco rattle virus* in Bleeding Heart [*Dicentra spectabilis* (L.) Lem.] Growing in Alaska

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*Tobacco rattle virus* (TRV) is a plant pathogen that is responsible for causing disease in many crop, ornamental, and weedy plants throughout the world (2). It is a member of the family *Virgaviiridae*, genus *Tobravirus*, and contains two positive single-stranded RNAs (1). RNA 1 is the largest strand that contains replicating capabilities, and may occur in nature as a NM-type isolate causing severe plant symptoms without the presence of RNA 2. NM-type isolates are probably not transmitted by TRV’s natural nematode vectors (*Paratrichodorus* and *Trichodorus* species) and is less likely to be experimentally sap-transmitted to known indicator test plants. The shorter RNA 2 contain the coat protein gene for complete particle integrity and necessary genes for nematode transmission, and requires the presence of RNA1 for infectivity; RNA1 and RNA2 together are known as M-type isolates.

In 2010 and 2011, oak-leaf patterns and ring spots on leaves of bleeding heart [*Dicentra spectabilis* (L.) Lem. (family *Papaveraceae*)] plants (Fig. 1) were found in several home gardens in south-central Alaska (Palmer and Wasilla, AK). Bleeding heart is a popular cold-hardy ornamental plant that is grown in Alaskan home gardens as a perennial for its heart-shaped flowers. Since these symptoms were similar for TRV-infected peony plants described in north-central Alaska (3), assays were implemented for definitive virus identification from four symptomatic bleeding heart plants identified as No. 1, -2, -3, and -4.

**Fig. 1.** Bleeding heart (*Dicentra spectabilis* L.) plants infected with *Tobacco rattle virus* that are (A) stunted, display (B) oak-leaf pattern, and (C) ringspots.
Isolation of virus occurred by homogenizing 1 gram of leaf tissue in citrate buffer, followed by two cycles of differential centrifugation, and final pellet resuspension in ~100 µl water. TRV confirmation was determined by detecting coat protein (CP) on SDS-PAGE mini-gels that were subsequently stained with Coomassie Blue or blotted onto a nitrocellulose membrane and exposed to TRV antiserum (AC Diagnostics Inc., Fayetteville, AR). Western blots clearly revealed a putative CP ~27kDa indicating that TRV was present for plants No. 1 and No. 3. Successful TRV transmission by sap and/or purified virus occurred with symptoms on *Nicotiana benthamiana* Domin, *Chenopodium amaranticolor* Coste. & Reyn., and *C. quinoa* Willd. (Fig. 2), and confirmation by reverse transcription (RT)-PCR (polymerase chain reaction) and Western blots for only plants No. 1 and No. 3.

Total RNA was extracted from the four bleeding heart plants with RNeasy Kit (Qiagen Inc., Valencia, CA) as directed, and used as a template for RT-PCR with primer set TRV-F (4). The gene (P1b) that codes for a 16kDa protein within the generated 515 bp segment was sequenced and deposited in GenBank from each of the four plants (accession numbers JX915802, JX915803, JX915804, and JX915805, respectively). Next, TRV-1 AKBH (Alaska bleeding heart) isolate from bleeding heart No. 1 was further scrutinized by using seven sets of overlapping primers (TRV-A through G) in RT-PCR that spanned TRV RNA1 (4). Fragments were sequenced, and assembled with the software program Sequencher 5.0 (Gene Codes Corp., Ann Arbor, MI). RNA1 from isolate TRV-AKBH contained 6,638 nucleotides that encode four non-structural proteins involved in replication and intercellular movement in the plant (GenBank
accession number JX912715). When aligned with six TRV RNA 1 isolates in GenBank, TRV-1 AKBH lacked an estimated 150 nts on the 5′-terminus. TRV-1 AKBH varied from 91% to 94% nucleotide identity among these isolates. Phylogenetically, TRV-1 AKBH RNA1 was most similar to the isolate TRV-AL from the ornamental alstroemeria (Fig. 3).

Fig. 3. Phylogram depicting nucleotide RNA1 relationships among seven Tobacco rattle virus (TRV) isolates (GenBank accession numbers) from alstroemeria, bleeding heart, potato, and spinach with Pepper ringspot virus (PepRSV) as an outgroup in the same genus Tobravirus; created with Clustal_X using pairwise alignments and 1000 bootstrap. Note that the Alaskan TRV isolate from bleeding heart (JX912715) is most closely associated with the isolate (HM195288) from another perennial ornamental plant, alstroemeria.
Although all four symptomatic *Dicentra* plants were determined to be infected with TRV by RT-PCR, two of the plants tested negatively by western blots, and failed to transmit TRV to indicator host plants. This information strongly suggests that TRV-M type was isolated from bleeding heart plants No. 1 and No. 3 whereas TRV NM-type isolates were derived from bleeding heart plants No. 2 and No. 4. It is obvious that RT-PCR is the best overall technique for TRV detection and definitive confirmation, detecting RNA1 from both M- and NM-types. Both M and NM-types of TRV may be readily spread by vegetative propagation of infected roots to new geographic regions. This research represents the first report of TRV in bleeding hearts from Alaska. The importation of vegetative propagated ornamentals such as *D. spectabilis* that harbor viruses, disperse viruses to new locations with possible natural spread to other susceptible plant species.

**Literature Cited**