

**Table S1.** Location and form of samples use in this study.

<b>Sample number</b>	<b>Sample type</b>	<b>Harvest location</b>	<b>Virus source</b>
S1	Field soil	Wilkin Cty., MN	Bait plants
S2	Field soil	Wilkin Cty., MN	Bait plants
S3	Field soil	Richland Cty., ND	Bait plants
S4	Field soil	Gooding Cty., ID	Bait plants
S5	Field soil	Jerome Cty., ID	Bait plants
S6	Sugar beet root	Renville Cty., MN	Root tissue

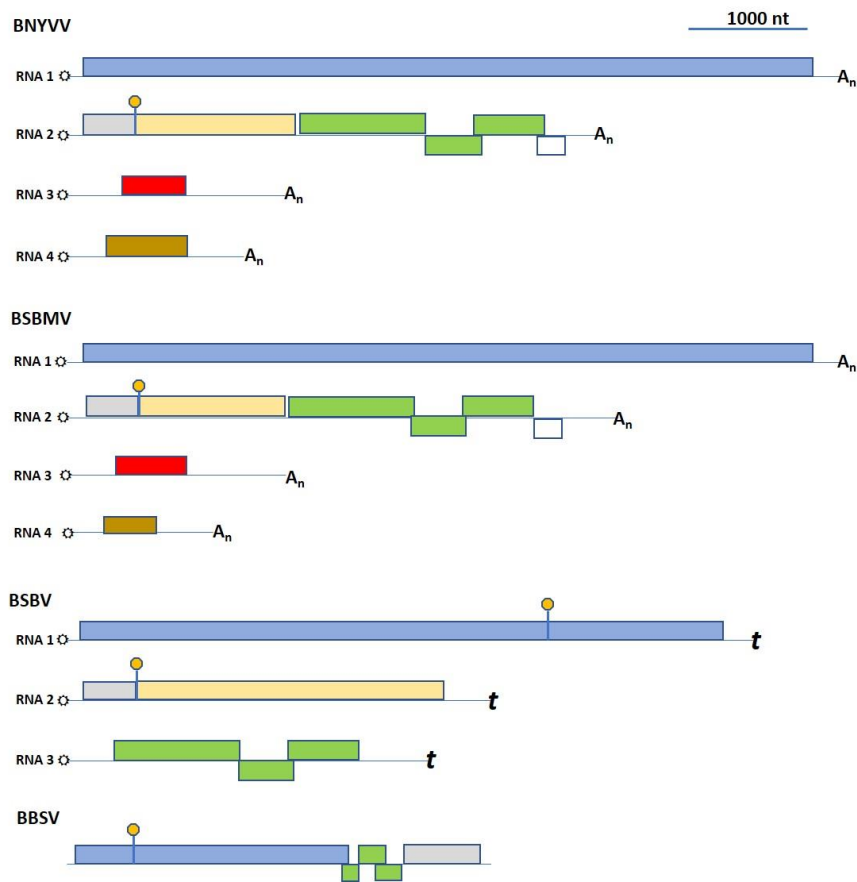
**Table S2.** Primers used in this study.

Primer name	Role	Sequence (5' – 3') <sup>a</sup>
MDB-2100	Alphanec P52 For	GTTTGGTATCACACCAGACCTTCAGGAAGCTCTGGAG
MDB-2101	Alphanec P30 Rev	GTTGATAGGTTCAATGAACTCTATCGCGTACTTG
MDB-1867	Satellite coat protein For	CAATACGAAATGGCAGGCTCAT
MDB-1868	Satellite coat protein Rev	ATCAAAGTAACGGACAGTACAA
MDB-2428	RNA 1 fragment 1 For	AAAAGGATCCTAATACGACTCACTATAGAAATTCGATTCTTC
MDB-2376	RNA 1 fragment 1 Rev	GTAAATCCACTTCAACAGCAAATTATACTTTGAAGAAG
MDB-2377	RNA 1 fragment 2 For	CTTCTTCAAAGTATAATTTTGCTGTTGAAGTGGATTTAAC
MDB-2378	RNA 1 fragment 2 Rev	TCGGAATAATGAATGATTTTAGTGTCTGGG
MDB-2379	RNA 1 fragment 3 For	CCCAGACACTAAAATCATTATTCCGA
MDB-2380	RNA 1 fragment 3 Rev	TTCCGCTTAAAATATATCTGAGCCGGGCCACACACATAC
MDB-2381	RNA 1 fragment 4 For	GTATGTGTGTGGCCCCGGCTCAGATATATTTTAAGCGGAA
MDB-2429	RNA 1 fragment 4 Rev	AAAAGGATCCAAGCTTTATATC
MDB-2460	Alphanec genome For	TAATACGACTCACTATAGAGTATWCATACCAAGWATACS
MDB-2462	Alphanec genome Rev	ATCATATGGGGTGGGGCAAARCCCCTCAATCTG

<sup>a</sup>Red: *Bam*HI restriction site

Orange: T7 promoter sequence

Green: PolyA of 60 nucleotides



**Figure S1.** General genome organization of dominant viruses detected through RNAseq applied to sugar beet roots. Lines represent the (+)-sense RNA genomes possessing either no distinguishing terminal features, as in BBSV, or possessing 5'-cap structures (⚙) and 3'-polyA tails ( $A_n$ ) or tRNA-like structures ( $t$ ). ORFs (boxes) are known or suspected to encode viral activities for replication (■), encapsidation (■), cell-to-cell movement (■), soilborne vector transmission (■), virus aggressiveness (■), and RNA silencing suppression (□). Suppression of a stop codon (●) to produce read-through proteins is a common theme in the expression of the genomes. Scaling bar = 1000 nucleotides (nt).

**A**

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BvSat1A      TTCTTTTCTT CACCAT TCACCA TAATAAAGRAAGAGRAACTACCTTAACCTATTGCA 56
              |||
BvSat1B      GTATTTCTT TTTCTT CACCAT TCACCA TAATAAAGRAAGAGRAACTACCTTAACCTATTGCA 60
Query 57      AGTTTACGCGCTAC CGACAGGTACGGGGGCGCA GTATTA CTTAOC GACAGG TATCGGAGTA 116
Sbjct 61      AGTTTACGCGCTAC CGACAGGTACGGGGGCGCA GTATTA CTTAOC GACAGG TATCGGAGTA 120
Query 117     GTATATTC TTGCAA TAGTTA CGTTTA TTGGAA CGTAAAGCTCA TATCGT TTTTAT GCTT 176
Sbjct 121     GTATACTC TTGCAA TAGTTA CGTTTA TTGGAA CGTAAAGCTCA TATCGT TTTTAT GCTT 180
Query 177     TT TGTGTT CCGGCT AAATTG TTTTGT TAATAA JCTTAC AATAGCA AATGG CAGGCT CATC 236
Sbjct 181     TT AGTTTT CCGGCT AAATTG TTTTGT TAATAA JCTTAC AATAGCA AATGG ----- 229
Query 237     AT CGAAGA GGGGTC AGTTTC CAATGC GTGGAG CGAAAAGCAGG GCGAAA TGATCA GCGG 296
Sbjct 230     -----C GCGGAG CAAAGAAACAGG GCGAAA TGATCA GCGG 264
Query 297     TCACAAGTAAGGC AAATGA TCAGGA GTCACTGACTTAC ATACTC GAGAA TGAAGA TGCG 356
Sbjct 265     TCACAAGTAAGGC AAATGA TCAGGA GTCACTGACTTAC ATACTC GAGAA TGAAAA TGCT 324
Query 357     AA CTTGCT TAAACA GTGGTACTTTGT ---TA GCTTCT GGAGCT ATTTGA GCATTACGA 412
Sbjct 325     GA CTTGCT TAAACG GTGGCAATCTAT CGATTA GC ---GCTGCC GTAGGT GGAATAACAA 380
Query 413     CGAATATT CCGCAA GAGATTAATGTC TCTGGA CGGACA GGGTAT CAGATA AATGTA CATT 472
Sbjct 381     GCACATC CAACAG GGTGATTAATGTT TCTGGA CGTACC GGGTAT CAAATAAATGTA CATT 440
Query 473     CTATCAAGATTTGGTTCAA GCTAGCATGAAT GCGCTT GCCAAT GTTGAT CGTTTT CGTT 532
Sbjct 441     CGGTCAAGATGTGGTTCAA GCTAGT ATGAA C GCGCTT GCCAAT GTTGAT CGTTTT CGTT 500
Query 533     ACATCTTA TTTTAT GATGCT CTTAAT ACAGST ACCCGG CCGGTA GTTCCA GATGTA CTTG 592
Sbjct 501     ACATTTTA TTTTAT GATGCT CTTAAT ACAGST ACCCCA CCAACG GTTGCT GATGTT TTAG 560
Query 593     AC ACTGCT TACGTT CTCTCT GATTAC TCATAC TTTGCA CGTACG CAGAAG AGTTCAAAT 652
Sbjct 561     AC ACTGCC GTTGTCT CTCTCT GACTAT GCGTAT TTTGCT CGTACA CAAAAC AGGTATAAGA 620
Query 653     TCCTTGTG GACAAACGACTT CACTATGCTAAT ACTGTA ATTTGG GAGGCT GTTAAT TATG 712
Sbjct 621     TTCTGCTT GATAAGCAGTCT CACTATGCTAAT AGCGGT AATTCT AGAGCT GTTAAT TACG 680
Query 713     AGCATGAGTTTAAA TTTAAGAACTACT CTGCTT GTGAT TTGTTGGGACC ACCCAACT 772
Sbjct 681     AGCATGAGTTTAAA TTTAAGAACTAAGGTTACC GTTGAT TGTTGGGAAACCAAGAAATC 740
Query 773     AC GCGCCT AATTCT TCTTTT TATTGATCATG GATGAT TTAGST TCTAAT AATAGT ADCT 832
Sbjct 741     AC GCGCCT AATTCT TTTTAT TATTGATCATG GATGAT TTAGST TCTAAT AATAGT ADCT 800
Query 833     AT GCGGTC AGTTGT ACTGTC CGTTAC TT TGGT GATTAA AAGCAT ATCTTC TTGTCC TTGG 892
Sbjct 801     AT GCGATC AGTTGT ACTGTT CGCTACT TT TGGT GATTAA AAGCAT ACCTTCT TTGTCC TTGG 860
Query 893     OGCGGTTT CTCAAC ACAGGAAAGAT GCGCCT TTTGTC TCGGCC GTTTTT AAACACAGCA 952
Sbjct 861     OGCGGTTT CTCAAC ACAGGAAAGAT GCGCCT TTTGTC TCGGCC -----CGC GCGC- 910
Query 953     GA CTTGTC GTTCTT CTCAAC CAAATC CGAGAA GGTGGG TACAAC GATATGT ACCAGT GCT 1012
Sbjct 911     -----G GATCT CTG-----CGTTGATACCAC-----TGCT 936
Query 1013    TA TGATTT CACTTA AATGAA TCGCTT GACATG TGTGCT AGTGGC TATCAA GATAAT TGCA 1072
Sbjct 937     T 937
Query 1073    AAAAACTT GATATT TGATAT 1094

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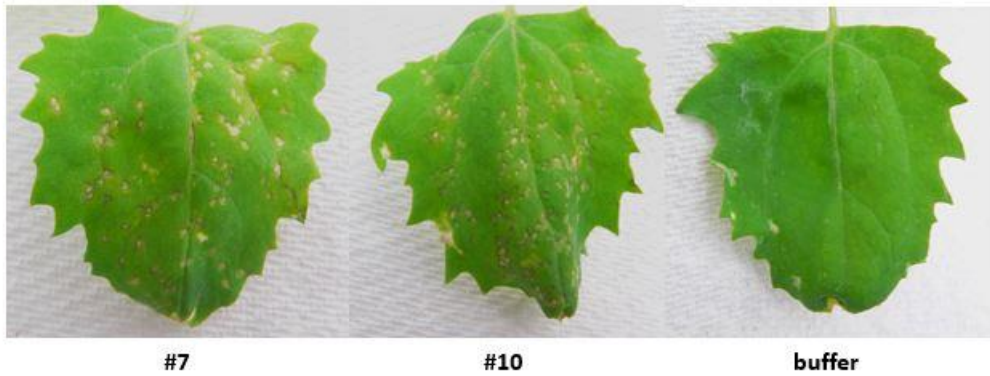
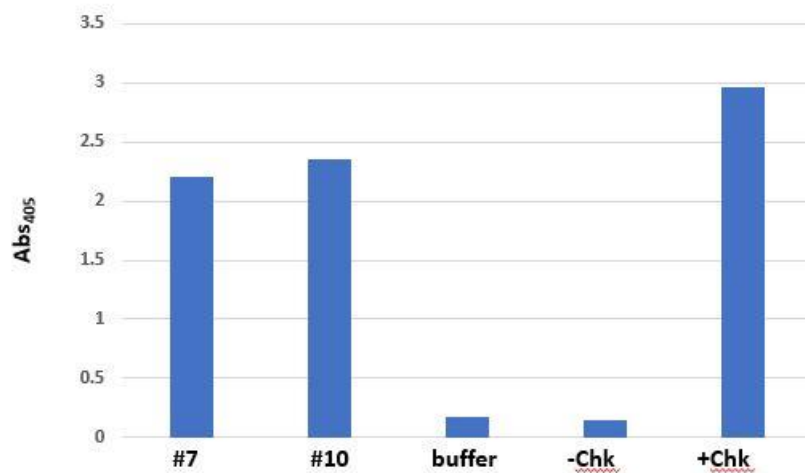
**B**

**unnamed protein product**  
Sequence ID: **Query\_62507** Length: **202** Number of Matches: **1**

Range 1: 1 to 202 [Genes](#) [View Match >](#) [Previous Match <](#)

Score	Expect	Method	Identities	Positives	Gaps
365 bits(938)	9e-136	Compositional matrix adjust.	172/202(85%)	186/202(92%)	0/202(0%)
<b>BvSat1A</b> 1		MRRAKKQGEHISRQQVRQIRSHDLATRELKMLTFVNGNLSISGAVGGITSNIQGQNV		60	
<b>BvSat1B</b> 1		MRRAKKQGEHISRQQVRQIRSHDLTRELKMPFVNSGTLASGAIQDITINIPQGNV		60	
<b>BvSat1A</b> 61		SGRTGVQINVHVSQWQFQASMNALANVDFRYILFYDRLNTGTPPTVADVLDTAVVISDY		120	
<b>BvSat1B</b> 61		SGRTGVQINVHS+K+HFQASMNALANVDFRYILFYDRLNTGTPPV+DVLDTA V+SDV		120	
<b>BvSat1A</b> 121		AVFARTQNRVYLLVDKTPVHSISGNSRAWNYEHFKFKNTVTVDSLGTTHNGRNSFYLL		180	
<b>BvSat1B</b> 121		+VFARTQNR+K LVDKT+PMSI+GNSRAWNYEHFKFKNTV VD LGTTN+GRNSF+YLL		180	
<b>BvSat1A</b> 181		VMGDLVSNNSYVAISCTVRVFD 202			
<b>BvSat1B</b> 181		+H DL NNSYVAISCTVRVFD 202			

**Figure S2.** Sequence analysis of the novel satellite virus genome discovered in sugar beet using RNAseq. **(A)** The two isoforms, present in roughly equal proportions in gel electrophoresis and abundance in the RNAseq read pool, are 72% similar at the nt level, with the size difference due to deletions in variant BvSat1B of sequence information present in BvSat1A. **(B)** An in-frame deletion in BvSat1B results in a truncated amino terminus of the predicted coat protein of this variant, lacking the first 12 residues present on the homologous protein of BvSat1A (see Figure 5B). Despite these differences, the level of similarity between the proteins of the two variants as predicted is 85% for that encoded by BvSat1A and BvSat1B.

**A****B**

**Figure S3.** Infection of *C. quinoa* with novel Alphanecrovirus synthetic RNA based on RNAseq genome data and detection of infecting virus by ELISA. (A) Expanded leaves of *C. quinoa* plants were inoculated with uncapped RNA transcribed from Clones #7 and #10. Necrotic lesions characteristic of members of the Alphanecroviruses emerged on the inoculated leaves at 5 dpi. (B) Infected leaves at 10 dpi produced source extracts for the detection of virus by ELISA using anti-TNV-A antiserum. Reduced levels of antigen detected in leaves inoculated with RNA from Clone-source #7 and #10, relative to that from TNV-A, may be due to differential virus accumulation between these clones and TNV-A (+chk) or to sequence variation between the novel virus and TNV-A in the CP (93% AA sequence similarity). The buffer-inoculated and healthy plant (-chk) controls provide the baseline for determining a positive reaction (positive > 3× baseline = 0.472 OD<sub>405</sub>).