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First Report of Tomato Ringspot Virus in *Phlox stolonifera* in the Netherlands

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In April 2023 a sample of creeping phlox (*Phlox stolonifera* cv. Purpurea; NPPO-NL 6175004) was submitted to the Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP), following an official report from an operator to the Dutch National Plant Protection Organization. The operator had conducted routine screening for various virus species using DAS-ELISA as a precautionary measure and detected tomato ringspot virus (ToRSV; *Nepovirus lycopersici*). At NIVIP, the plant was visually inspected but did not exhibit any symptoms (Figure 1). To confirm the operator's findings, a leaf sample was re-tested for ToRSV using DAS-ELISA (Prime Diagnostics, The Netherlands) in accordance with PM 7/125 (European and Mediterranean Plant Protection Organization 2025) and yielded a positive result. Furthermore, mechanical transmission tests were performed using four herbaceous indicator plant species, following the method described in PM 7/153 (European and Mediterranean Plant Protection Organization 2022). The inoculation resulted in infections in *Chenopodium quinoa* (local symptoms), *Nicotiana benthamiana* (systemic symptoms; Figure 2A), *N. occidentalis* P1 (local and systemic symptoms; Figure 2B,C) and *N. tabacum* cv. White Burley (local symptoms).

To verify the identity of the virus, high-throughput sequencing was conducted as described by Giesbers et al. (2023): Ribosomal RNA-depleted 150 paired-end Illumina data was generated from total RNA extracted from 1 g of phlox leaf tissue using an RNeasy Plant Mini Kit (Qiagen, The Netherlands). Two gigabases were analysed using a custom workflow for detection and identification of *de novo* assembled viral contigs. Two contigs showed significant similarity to viral genomes and corresponded to two ToRSV genome segments: RNA1 (8149 nt; 43,856 mean read depth (MRD)) and RNA2 (6920 nt; 104,148 MRD) both encoding one protein. Near-complete genome sequences were submitted to GenBank (Accession Nos. PV288328 and PV288329), with 81.8 and

FIGURE 1 | *Phlox stolonifera* cv. Purpurea asymptomatically infected with tomato ringspot virus.

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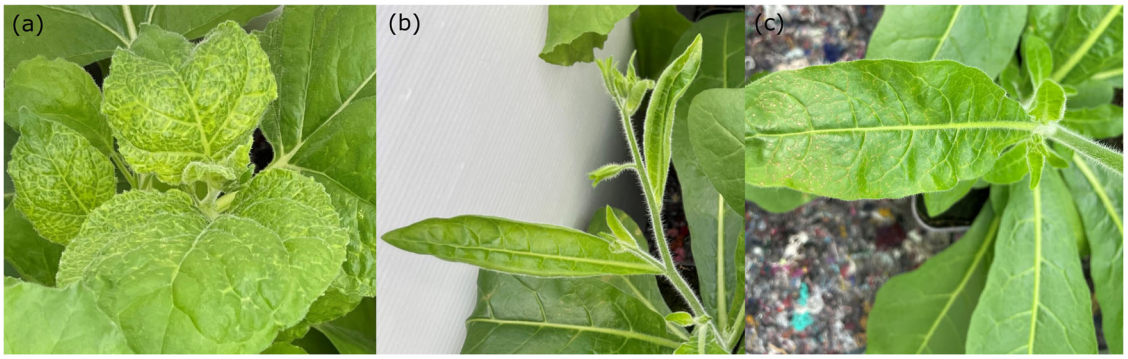

FIGURE 2 | Symptom expression after mechanical inoculation of tomato ringspot virus: (a) *Nicotiana benthamiana* displaying systemic vein clearing and stunted growth, (b) *N. occidentalis* P1 displaying systemic leaf curling and growth reduction and (c) *N. occidentalis* P1 displaying systemic necrotic lesions.

74.4% nucleotide identity to the reference sequences NC_003840 (RNA1) and NC_003839 (RNA2), respectively. The metagenomic sequence data were also deposited under BioProject Accession No. PRJNA1234858. The virus isolate has been deposited in the Leibniz Institute DSMZ collection with collection number PV-1438.

To our knowledge this is the first report of ToRSV in *Phlox stolonifera* worldwide. The lack of visible symptoms in the ToRSV-infected plant is consistent with a pattern observed in other vegetatively propagated ornamentals, such as *Iris germanica* and *Pelargonium* (Department for Environment, Food, and Rural Affairs 2018; European and Mediterranean Plant Protection Organization 2018). Similar asymptomatic infections have been reported in ornamentals for another nepovirus, tobacco ringspot virus (TRSV), across multiple European countries as described by Schoen et al. (2024). These findings have contributed to changes in the phytosanitary status of TRSV and ToRSV within the European Union, reclassifying them from quarantine pests to regulated non-quarantine pests. This finding of ToRSV highlights the need for further surveillance efforts, with a focus on asymptomatic vegetatively propagated ornamentals, to gain insight into the possible asymptomatic presence of viruses with phytosanitary significance.

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