Association of *Tobacco ringspot virus*, *Tomato ringspot virus* and *Xiphinema americanum* with a decline of highbush blueberry in New York

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Abstract

Plantings of highbush blueberry cultivars 'Patriot' and 'Bluecrop' showing virus-like symptoms and decline in vigor in New York were surveyed for the occurrence of viruses. *Tobacco ringspot virus* (TRSV) and *Tomato ringspot virus* (ToRSV) from the genus *Nepovirus* in the family *Comoviridae* were identified in leaf samples by DAS-ELISA. Their presence was confirmed by RT-PCR with amplification of 320-bp and 585-bp fragments of the RNA-dependent RNA polymerase genes, respectively. Comparative sequence analysis of viral amplicons of New York isolates indicated moderate (80.7-99.7 %) to high (90.8-99.7 %) nucleotide sequence identities with other ToRSV and TRSV strains, respectively. Soil samples from the root zone of blueberry bushes contained dagger nematodes and cucumber bait plants potted in soil samples with identified *X. americanum* became infected with ToRSV or TRSV. Altogether, ToRSV, TRSV, and their vector *X. americanum sensu lato* are associated with the decline of highbush blueberry in New York.

Keywords: *Vaccinium corymbosum* L., dieback, DAS-ELISA, RT-PCR, RNA-dependent RNA polymerase gene

Introduction

In the spring of 2007, decline and virus-like symptoms were observed in plantings of mature highbush blueberry (*Vaccinium corymbosum* L.) cvs. 'Patriot' and 'Bluecrop' in New York. Symptoms consisted of stunted growth, top dieback or mosaic and dark reddish lesions on apical leaves. Necrosis of leaf and flower buds and reduced fruit yield were also noticed. These observations prompted a survey of highbush blueberry cvs. 'Patriot' and 'Bluecrop' for the occurrence of viruses and their vectors.

Materials and methods

Plantings of highbush blueberry cv. 'Bluecrop' and 'Patriot' were surveyed for the occurrence of viruses by double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) with specific antibodies (Bioreba, Reinach, Switzerland).

The presence of viruses identified by DAS-ELISA was confirmed by reverse transcription (RT) polymerase chain reaction (PCR) using total RNA extracted from leaf tissue and appropriate primers. RT-PCR was carried out using the OneStep kit (Qiagen, Valencia, CA) with a 30 min heating step at 50 °C and a 15 min heating step at 95 °C followed by 35 cycles of 30 sec melting at 94 °C, 1 min annealing at 50°C, and 1 min elongation at 72 °C with a final extension of 10 min at 72 °C. The reaction products were resolved by electrophoresis in 1.5 % agarose gels.

Viral DNA amplicons obtained by RT-PCR were extracted from agarose gels and sequenced bidirectionally. Sequences were analyzed and compared using the DNASTAR Lasergene® v7.2 software package and TRSV strain bud blight (GenBank accession no. U50869) and ToRSV strain raspberry (GenBank accession no. L19655**)** as reference strains.

Soil samples were collected from the root zone of blueberry bushes and analyzed for the occurrence of dagger nematodes, which were identified based on morphological parameters (Lamberti et al., 2002) and counted under a dissecting microscope at 40X magnification. Blueberry soil samples collected from the root zone of blueberry bushes were used in cucumber baiting assays in a greenhouse.

Results

Decline and virus-like symptoms were observed in plantings of mature highbush blueberry cvs. 'Patriot' and 'Bluecrop' in New York in the spring of 2007. Symptoms in cultivar 'Patriot' consisted of a severely reduced vigor, shoot defoliation, distorted leaves and chlorosis of apical leaves, vein clearing or mosaic. Some bushes also showed a top dieback with poor blossom development or necrotic flower buds, and a few of them were dead. The decline symptoms were observed throughout the planting of blueberry cv. 'Patriot'. Symptoms in cultivar 'Bluecrop' consisted of mosaic or dark reddish lesions on apical leaves and a general decline.

Preliminary DAS-ELISA results indicated the presence of *Tomato ringspot virus* (TRSV) and *Tomato ringspot virus* (ToRSV) in a few leaf samples surveyed in the fall of 2007. Since these tests were run late in the season, an extensive

survey was conducted in the spring of 2008 to confirm preliminary findings and determine the incidence of these two virus species from the genus *Nepovirus*, family *Comoviridae* in every bush of the two blueberry plantings. Thirty-seven of the 528 blueberry leaf samples (7 %), including 439 from cv. 'Patriot' and 89 from cv. 'Bluecrop', reacted positively to ToRSV (3 %, 17 of 528) or TRSV (4 %, 20 of 528) in DAS-ELISA. Nine ToRSV-infected samples were from Patriot (2 %) and eight from Bluecrop (9 %); twelve Patriot (3 %) and eight Bluecrop (2 %) samples were infected with TRSV. The two target viruses were found in symptomatic and asymptomatic blueberry bushes, suggesting no strict association between virus detection by DAS-ELISA and symptom development. Seven soil samples from the Patriot planting and three from the Bluecrop planting were tested for the presence of nematodes. Species identified were primarily *Pratylenchus* spp., *Helicotylenchus* spp. and *Hoplolaimus* spp. but members of the *Xiphinema americanum* group were also found. *X. americanum* populations varied from 40 to 280 per kg of soil collected and were all from the root zone of Patriot bushes. Cucumber baiting assays with each of the four soil samples containing *X. americanum* demonstrated transmission of TRSV or ToRSV.

The presence of TRSV was confirmed by RT-PCR in blueberry leaf samples with amplification of a 320-bp DNA fragment of the RNA1-encoded RNA-dependent RNA polymerase (RdRp) using primers MF05-21-R (5' CAATACGGTAAGTGCACACCCCG 3') and MF05-22-F (5' CAGGGGCGTGAGTGGGGGCTC 3'). Similarly, the presence of ToRSV was confirmed in blueberry leaf tissue by immunocapture RT-PCR and primers ToRSV-R (5' CCACCACACTCCACCTACC 3') and ToRSV-F (5' ACTTCTGAAGGCTACCCGTT 3') to characterize a 585-bp fragment of the RNA1-encoded RdRp gene.

The viral gene amplicons obtained by PCR-based assays from four ToRSV and eleven TRSV isolates were sequenced. A multiple sequence alignment of ToRSV isolates from blueberry characterized in this study and those available in GenBank indicated 80.7-99.7 % and 90.2-99.5% sequence identity at the nucleotide (585 nts) and amino acid (194 residues) levels, respectively. Phylogenetic analyses showed a clustering of ToRSV isolates into three groups. Notwithstanding, ToRSV haplotypes from blueberry did not group with corresponding haplotypes from other crops, suggesting a genetic differentiation according to host. A multiple sequence alignment of TRSV isolates from blueberry characterized in this study and those available in GenBank indicated 90.8-99.7 % and 91.4-100 % sequence identity at the nucleotide (316 nts) and amino acid (105 residues) levels, respectively. Phylogenetic analyses inferred a clustering of TRSV isolates into a single group.

Discussion

ToRSV and TRSV are known to occur in fruit crops in New York, including grapevines (Gilmer et al., 1970; Uyemoto et al., 1977a) and *Prunus* spp. (Cummins and Gonsalves, 1986; Uyemoto et al., 1977b). ToRSV is also described in *Malus* spp. (Rosenberger et al., 1989) but, to our knowledge, this is the first report on the occurrence of TRSV and ToRSV in highbush blueberry in New York. In the USA, TRSV was reported in blueberry in Connecticut, Illinois, Michigan, Arkansas, Oregon and New Jersey (Ramsdell, 1985a). Also, ToRSV was described in blueberry in Oregon, Washington and Pennsylvania (Ramsdell, 1985b). The distribution of the two nepovirus species in the major blueberry producing areas in the United States is likely explained by the use of noncertified planting material.

The infection rate of TRSV and ToRSV was low in apical leaves in spite of a severe degeneration in cv. 'Patriot' and a slow decline in cv. 'Bluecrop'. Highbush Bluecrop is known to recover from TRSV infection (Lister et al., 1963). This recovery phenomenon likely results from the manifestation of RNA silencing. Interestingly, a complete or partial recovery of *N. tabaccum* and *N. benthamiana* from TRSV infection was described recently (Siddiqui et al., 2008) as well as a recovery of *N. benthamiana* from ToRSV infection (Jovel et al., 2007). It is conceivable that RNA silencing could be active in highbush blueberry cvs. 'Patriot' and 'Bluecrop' infected with TRSV or ToRSV, affecting systemic spread, reducing virus titers in apical leaves, in spite of a marked decline. *X. americanum*, the nematode vector of TRSV and ToRSV, was detected in the root zone of highbush blueberry cv. 'Patriot'. Populations of *X. americanum* were described in vineyards (Uyemoto et al., 1977a) and in apple, peach and cherry orchards (Molinari et al., 2004) in New York. To our knowledge, this is the first report of the dagger nematode *X. americanum senso lato* in blueberry in New York. The population density of *X. americanum* in the blueberry plantings surveyed in this study was low, but similar to findings in other crops (Evans et al., 2004; Pinkerton et al., 2008). In spite of the low population density detected, it is conceivable that *X. americanum* could contribute to the decline of highbush blueberry cvs. Patriot and Bluecrop by feeding on actively growing rootlets, thus weakening the root system and affecting plant vigor.

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