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Tomato ringspot nepovirus (ToRSV) in wild blackberry (*Rubus fruticosus* L.) in Hatay province of Turkey

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Abstract

During observations of virus-like symptoms in wild blackberry (*Rubus fruticosus* L., Rosaceae) some stunted plants growing in the border of stone-fruit orchards in Hatay were found showing severe yellow blotching and deformity of the leaves. Samples (shoots and leaves) were collected in September 2008 and May 2009 from wild blackberry plants growing at the border of apricot orchards and neighboring stone fruit nurseries in Hatay province in Eastern Mediterranean Region of Turkey. Each of 12 wild blackberry samples taken from 7 symptomatic and 5 symptomless plants were tested for virus by mechanical inoculation of sap to herbaceous plants. Sap was inoculated on *Chenopodium amaranthicolor*, *C. quinoa*, *Cucumis sativus*, *Gombhrena globosa* L., *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, *Phaseolus vulgaris* L. and *Vigna unguiculata* L. Sap from six symptomatic plants induced symptoms of necrotic or chlorotic lesions, and ringspot on test plants. No symptoms were induced in the test plants inoculated by sap from symptomless blackberry plants. A sap-transmissible virus was obtained from all symptomatic plants and later identified as *Tomato ringspot nepovirus* (ToRSV) by enzyme-linked immunosorbent assay-ELISA. Thus, results of biological indexing were also confirmed by serological assays (ELISA). Cuttings of symptomatic plants were rooted in pots and kept in an insect-proof growing room for symptom observations and testing. Investigations on the other viruses in wild and cultivated *Rubus* spp. and its vector/s are still in progress. Further studies are necessary to investigate the distribution and natural transmission of the main virus diseases in cultivated *Rubus* spp. in particular because of the economic importance of *Rubus* cultivation and the recent increase in new commercial plantings in Hatay. This work represents the first report of ToRSV in wild blackberry (*R. fruticosus*) in Turkey.

Keywords: Bioassay, Blackberry, ELISA, *Rubus*, ToRSV, virus

Introduction

Blackberries have been reported to be infected by several viruses (Spak, 1995; Martin et al., 2004; Tzanetakis and Martin, 2004; Tzanetakis et al., 2008). *Tomato ringspot virus* (ToRSV) is one of the more important viruses causing diseases in *Rubus* spp. ToRSV is a distinctive member of the genus *Nepovirus* (*Comoviridae*) (Stace-Smith, 1996). The virus is found in woody and semi-woody hosts, but it can also be found in herbaceous ornamental and weed species. ToRSV has a similar host range to *Tobacco ringspot nepovirus* (TRSV) (OEPP/EPPO, 2001). It also causes infection in main fruit crops including *Prunus* spp. which are important in the Eastern Mediterranean Region of Turkey. Blackberries (*Rubus* sp.) are also becoming an economically and socially important crop in Hatay province which is located in the same region.

Although *Rubus* stunt disease in *Rubus* spp was reported for the first time in Turkey (Sertkaya et al., 2004), there is lack of knowledge about virus diseases of blackberries in Turkey. The aim of this study is to investigate *Tomato ringspot nepovirus* (ToRSV) on wild blackberry (*Rubus fruticosus* L., Rosaceae) in the Hatay province of Turkey.

Material and methods

Field observations were made for the identification of symptoms on plants growing in the border of commercial stone fruit orchards in the Hatay province of the Eastern Mediterranean Region of Turkey in September 2008 and May 2009. In order to determine the presence of *Tomato ringspot nepovirus* (ToRSV) on wild blackberry (*Rubus fruticosus* L.), visual inspections were made. A total of 12 shoot and leaf samples were collected from 7 plants exhibiting severe symptoms and 5 asymptomatic plants.

Shoot and leaves samples were collected from the same plants and assayed with sap inoculations and the Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) in both autumn and spring. Mechanical transmission of the virus was carried out with the herbaceous test plants *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, *Phaseolus vulgaris* L. and *Vigna unguiculata* L. Young blackberry leaves were homogenized in 0.1 M phosphate buffer (pH 7.2) containing 2% nicotine in a pestle and mortar, and the sap extracts rub-inoculated onto Celite-dusted leaves of the herbaceous indicator plants. Four plants from each of the herbaceous indicator species were mechanically inoculated with the sap of a single blackberry sample. All samples taken from wild blackberry and inoculated test plants were tested for the presence of ToRSV by ELISA as described by Clark and Adams (1977). Antiserum kits from Bioreba AG (Switzerland) were used in standard DAS-ELISA. Each group including four indicator plants of each species used for testing for each sample in Bioassays were accepted as one sample for ELISA.

Small cuttings were excised from investigated symptomatic plants, rooted in pots containing a peat:turf (1:1) mixture and kept in insect-proof growing room at 25°C±2 with a 16:8 h photoperiod (day:night) in the autumn of 2008 for symptom observation and indexing.

Results and discussion

During the field inspections, some virus-like symptoms were observed on wild blackberry plants growing in the border of stone fruit orchards in Hatay. Suspicious blackberry plants mainly exhibited symptoms possibly related to disease in *Rubus* spp. caused by *Tomato ringspot nepovirus* (ToRSV) such as stunting, deformity of the leaves, severe yellow blotching or chlorosis, and at the end of autumn the chlorotic areas became an intense yellow (Figure 1). ToRSV can be transmitted by nematode vector, *Xiphinema* spp. (Brown, 1989).

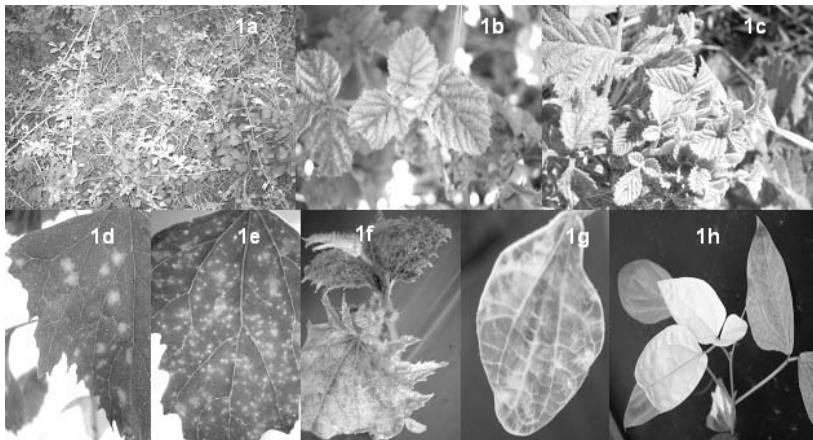


Figure 1 Symptoms on plants infected by *Tomato ringspot virus* (ToRSV); Wild blackberry: stunting, deformity of the leaves, severe yellow blotching or chlorosis on *Rubus fruticosus* L. (1a-c), Test plants: Chlorotic local lesions were developed on test plants, *C. amaranticolor* (1d), *C. quinoa* (1e), *Cucumis sativus* (1f), *P. vulgaris* (1g) and *V. unguiculata* (1h), in two weeks after sap inoculations.

However, no obvious symptoms of ToRSV were observed on peach plants in orchards close to sampled wild blackberry plants. A total of 12 samples taken from 7 symptomatic and 5 asymptomatic plants were tested for virus by mechanical inoculation of sap to herbaceous test plants. Chlorotic local lesions developed in *C. amaranticolor*, *C. quinoa*, *P. vulgaris* and *V. unguiculata* test plants two weeks after sap inoculation: necrotic lesions in *C. quinoa*, mosaic in *C. sativus*, and rugosity in *P. vulgaris* (Figure 1 and Table 1).

Table 1 Symptomatology of host plants mechanically inoculated by sap of wild blackberry (*Rubus fruticosus* L., Rosaceae)

Indicator Plants	Symptoms
Family: Amaranthaceae	
<i>Gomphrena globosa</i> L.	0
Family: Chenopodiaceae	
<i>Chenopodium amaranticolor</i> Coste and Reyn	C. L.L.
<i>Chenopodium quinoa</i> Wild	C. L.L., (or N.L.L.)
Family: Cucurbitaceae	
<i>Cucumis sativus</i> L. cv. Cemre F1	M, Cl
Family: Fabaceae	
<i>Phaseolus vulgaris</i> L.	C.L.L. (or Ru.)
<i>Vigna unguiculata</i> L.	C.L.L
Family: Solanaceae	
<i>Nicotiana benthamiana</i> L., <i>Nicotiana clevelandii</i> L.	0
<i>Nicotiana glutinosa</i> L.	0

C.L.L.=Chlorotic Local Lesion, Cl.=Chlorosis, Ru.=Rugosity, M=Mosaic, N.L.L.=Necrotic, 0=No symptoms.

Sap from six symptomatic wild blackberry plants induced symptoms of chlorotic or necrotic lesions, rugosity on test plants. No symptoms were induced in the test plants inoculated by sap from one symptomatic and five symptomless blackberry plants. Serological tests are necessary to identify specifically the reactions produced on indicator plants. However, leaf samples collected from the all inoculated test plants including asymptomatic ones were also analysed by DAS-ELISA. A sap-transmissible virus was identified as *Tomato ringspot virus* (ToRSV) in six out of seven symptomatic plants, but not from five symptomless plants, by sap transmission and ELISA tests. The results were confirmed by re-testing in May 2009. One symptomatic sample might have been infected with different isolates of ToRSV or another virus which causes similar symptoms on the blackberry plants.

Seventeen of 25 cuttings survived and developed symptoms of stunting, chlorosis and deformity of the leaves on new shoots in May 2009. Of 11 rooted cuttings that were detected by ELISA, 8 were infected with ToRSV. ELISA results confirmed the presence of ToRSV in wild *Rubus* spp. in our region.

Investigations into the distribution and transmission of ToRSV on cultivated *Rubus* spp. by grafting and detection of potential vector nematode species such as *Xiphinema* spp. around the roots of symptomatic plants are in progress. Further studies are also necessary to investigate the present status of virus diseases in cultivated *Rubus* spp. in Turkey. Further studies are necessary to determine the distribution and natural transmission of the main virus diseases in cultivated *Rubus* spp. especially due to the economic importance of rubus cultivation in Hatay.

This work represents the first report of ToRSV in wild blackberry (*R. fruticosus*) in Turkey.

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