Pome fruit viruses in Bosnia and Herzegovina

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Abstract

During autumn 2005 and summer 2006, field surveys were carried out to assess the sanitary status of pome fruit trees in Bosnia and Herzegovina. Inspections were done in the main pome fruit growing areas including 10 orchards, 2 nurseries and one varietal collection. A total of 65 apple and 50 pear cultivars were tested by biological indexing for the presence of *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV) and *Apple mosaic virus* (ApMV). The average infection level was 81%. Both species showed a similar infection rate (83% for apple and 78% for pear). The most frequent viruses of apple were ACLSV (72%) and ASPV (69%), and of pear ASGV (69%) and ACLSV (64%). The same samples were also tested by ELISA, with a lower virus detection rate compared to the biological indexing. Multiplex RT-PCR results of 20 randomly selected apple cultivars were in line with biological indexing. Results of our surveys report for the first time the presence of ACLSV, ASPV, ASGV and ApMV on pome fruits in Bosnia and Herzegovina.

Keywords: *Malus*, biological indexing, ELISA, multiplex RT-PCR, sanitary status

Introduction

Pome fruit growing in Bosnia and Herzegovina is practiced mainly in the northern part of the country. Among the pome fruits, apple and pear are the most important crops. There are 8,000 ha of cultivated apple and 2,600 ha of pear (Anonymous, 2002). The most popular cultivars are 'Idared', 'Golden Delicious', 'Granny Smith' and 'Jonagold', grafted mostly on the rootstock MM106 and less on M9. The leading pear cv. is 'William' and common rootstocks for pear are quince and pear seedlings. Pome fruit viruses are frequently latent and therefore easily spread by propagation material. Latent viruses are more widespread than the others (Hadidi, et al., 2003). The main viruses affecting pome fruit trees are *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV) and *Apple mosaic virus* (ApMV). These viruses occur frequently in mixed infections and can cause significant yield reduction (Posnette et al., 1963; Desvignes, 1999). This study, the results of which are presented here, set out to survey for the presence of pome fruit viruses, for the first time, in Bosnia and Herzegovina.

Materials and methods

Surveys for symptoms and sample collections were done in the main pome fruit growing regions. Samples were collected from 10 commercial orchards, 2 nurseries and one varietal collection. The visited commercial orchards were of different sizes and mostly young, though a few older orchards were also visited. The varietal collection was mid-aged, but not properly managed, in particular for pest control practices. Most of the visited pome fruit trees did not show any symptoms and that is in line with previous reports on the latency of pome fruit viruses (Desvignes, 1999). However, in some orchards, leaf symptoms of yellow mosaic on apple cvs. 'Idared', 'Jonagold', 'Granny Smith' and 'Melrose', associated with the presence of ApMV, and vein yellowing on pear cultivars: 'Mindjusica', 'Kolacusa' and 'Sarajka', associated with a strain of ASPV, were observed. Bud-sticks of 30 to 40 cm long from one-year old shoots were taken from 115 trees of pome fruits (65 apple and 50 pear les, each of them a different cultivar) during autumn 2005, whereas symptom observation was done during spring-summer 2006. Collected budwoods were grafted onto one–year old virus-free seedlings of indicators of apple (Spy 227, R 12740 7A and 'Virginia Crab') and pear (*Pyronia veitchii*, LA 62 and 'Virginia Crab'). The entire collection was double chip-bud grafted using three indicator replicates per sample. Seven to ten days later, the rootstock was cut off 1-2 cm above the upper bud graft to force growth of the indicator. All inoculated plants were maintained in the acclimatized greenhouse at 20-24ºC and constant light. Grafting of indicator plants was done during December 2005. The first observation for symptoms was made 4 months after inoculation. Two more observations were done in May and June 2006.

All samples were tested by DAS-ELISA (Clark and Adams, 1977) for ApMV, ASPV and ASGV, and DAS-simultaneous ELISA (Flegg and Clark, 1979) for ACLSV. Serological reagents were provided by commercially kits (Loewe, Germany). For ASPV, ELISA was performed with Bioreba commercial kit (Switzerland). ELISA was done using leaf extracts from grafted indicator plants. Multiplex RT-PCR, for the simultaneous detection of ACLSV, ASPV, ASGV and ApMV, was done as described by Hassan et al. (2005). Total nucleic acid (TNA) extraction was according to Foissac et al. (2001). The PCR mixture contained a cocktail of five primer pairs: 1 μM for each ACLSV primer (Menzel et al., 2002), 0.8 μM for each ASPV primer (Menzel et al., 2002), 0.6 μM for each ASGV primer (Menzel et al., 2002), 0.4 μM for each ApMV primer (Hassan et al., 2005) and 0.25 μM for each internal control (*nad5*) primer (Menzel et al., 2002).

Results

- *R 12740 7A* - chlorotic spots and leaf deformation (small, sickle-shaped leaves) were seen, similar to those caused by ACLSV. Dwarfing of indicators grafted with apple cvs. 'Golden Delicious', 'Florina', 'Srcika' and 'Pinova' was also present. A total of 44 plants were infected out of 61 tested (infection rate 72%) for ACLSV (Table 1).
- *Spy 227* - epinasty of the leaves, showing within 2-3 months, was associated with the presence of ASPV. A total of 45 plants out of 65 tested (69%) were found infected with ASPV (Table 1).
- *Virginia Crab* – a necrotic line at graft union was associated with ASGV. A total of 10 plants, out of 30 tested (33%), were found infected by ASGV (Table 1). The results of this test were not satisfactory because a limited number of tests were considered reliable, due to the low graft take and the short observation period of 6 months after grafting. Biological testing of ASGV gives more reliable results in the open field in 2-3 years (Boscia et al., 1999).
- *LA 62* - chlorotic spots on the leaves were associated with the presence of ACLSV. Considered as sensitive indicator for the virus (Boscia et al., 1999), a total of 32 LA62 plants out of 50 tested (64%), were found to be infected (Table 1).
- *Pyronia veitchii* - epinasty and vein yellowing on the leaves were associated with the presence of PVYV (ASPV). A total of 11 plants were found infected out of 50 tested (22%) (Table 1).
- *V. Crab* - greenhouse tests indicated an infection rate of 69% by ASGV, due to 11 plants infected out of 16 tested. Numerous indicator plants (34 cultivars) were eliminated as non-tested (NT) due to the unsuccessful graft take (Table 1).

ELISA showed to be less reliable than biological indexing, which could be due to the host species, low virus titer, inhibitory effects of polysaccharides and phenolic compounds, as previously reported (Desvignes et al., 1992; Boscia et al., 1999; Kinard et al., 1996).

Tab. 1 Detected viruses by biological indexing and ELISA in apple and pear

	ACLSV		ASPV/PVYV		ASGV		ApMV	
Samples	**Index* (I/T)**	**ELISA (I/T)**	**Index** (I/T)**	**ELISA (I/T)**	**Index*** (I/T)**	**ELISA (I/T)**	**Field Sympt (I/T)**	**ELISA (I/T)**
Apple cultivars	44/61	13/61	45/65	9/65	10/30	1/30	4/65	0/65
Pears cultivars	32/50	12/50	11/50	6/50	11/16	1/16	0/50	0/50
Total	76/111	25/111	56/115	15/115	21/46	2/46	4/115	0/115

I: Infected; T: Tested; Woody indicators for apple: * R12 (ACLSV); **Spy 227 (ASPV); *** V. Crab (ASGV);
Woody indicators for pear: * LA62 (ACLSV); ***Pyronia veitchii* (PVYV); *** V. Crab (ASGV)

Thirty-five (30% of 115 samples) tested positive for at least with one virus(ACLSV, ASPV and ASGV). ApMV was not found (Table 1). Twenty randomly selected apple cultivars were tested by multiplex RT-PCR which detected the presence of all four viruses in apple (ACLSV, ASPV, ASGV and ApMV), significantly reducing the time required for detection. Amplified products of TNA extractions from leaves of indicator plants, showed clear bands of the expected size. Multiplex RT-PCR proved more sensitive (infection rate higher with 50%) as compared with ELISA. Interestingly, multiplex RT-PCR results were generally in line with those of biological indexing. Both techniques showed similar levels of infection rates for: ACLSV, ASPV and ASGV (data not shown).

Discussion

This is the first extensive survey on the sanitary status of pome fruit trees in Bosnia and Herzegovina including 115 cultivars: 65 apples and 50 pears. The present study showed that the local pome fruit industry in the country is affected by the presence and high incidence of apple and pear viruses. The presence of 4 viruses (ACLSV, ASPV, ASGV and

ApMV) was detected. The most frequent virus was ACLSV in apple and ASGV in pear. Latent infection by pome fruit viruses and their presence in nurseries present a threat to pome fruit production increasing the difficulty of their future control. To our knowledge, this is the first report of ACLSV, ASPV, ASGV and ApMV on pome fruits in Bosnia and Herzegovina.

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