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SHORT NOTES

A preliminary account on the sanitary status of stone fruits at the Clonal Genebank in Harrow, Canada

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Summary. Field observations and laboratory tests were carried out in Harrow to evaluate the sanitary status of the Clonal Genebank collection of stone fruit. The presence of viruses and viroids was determined by ELISA, tissue-printing hybridization and GF305 woody indexing. A total of 645 trees (197 peach and nectarine, 183 sweet and sour cherries, 106 plum, 106 apricot, and 53 other cherries) were tested by ELISA for the presence of *Plum pox virus* (PPV), *Prunus necrotic ring spot virus* (PNRSV) and *Prune dwarf virus* (PDV). No evidence of PPV infection was found in the collection. PNRSV and PDV were frequently detected in single and mixed infections. The overall average of virus infection rate was 20.3%. A total of 336 trees (116 peach and nectarine, 84 sweet and sour cherries, 54 plum, 44 apricot, and 38 other cherries) were tested by tissue printing hybridization for the presence of *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd). Thirty samples were infected, 28 peaches and nectarines with PLMVd and 2 apricots with HSVd. This is the first report to date, of HSVd presence in Canada. Finally, 114 (38.4%) out of 297 tested accessions were found infected with at least one virus and/or viroid.

Key words: PPV, ilarviruses, stone fruit viroids, ELISA, tissue printing hybridization.

Introduction

Plum pox virus (PPV) is the agent of Sharka disease,which causes heavy losses in peach, nectarine, apricot, cherry, and plum around the world. The virus was identified in Canada for the first time in 2000 (Thompson *et al*., 2001), specifically in Ontario and Nova Scotia, during a survey conducted by the Canadian Food Inspection Agency. The Clonal Genebank located in Harrow, Ontario, is the repository for tree fruit and small fruit species in Canada. Subsequent to the discovery and spread of PPV in Ontario, a project was devised to screen all Genebank material for the presence of PPV by ELISA and by indexing on GF305. Furthermore all Genebank *Prunus* accessions were screened for two pollen-transmitted viruses, *Prunus necrotic ring spot virus* (PNRSV) and *Prune dwarf virus* (PDV), by ELISA; and two viroids, *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd), by molecular hybridization. In this paper are reported the results of this screening.

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Materials and methods

Clonal Genebank

The Clonal Genebank in Harrow holds approximately 300 accessions of the following stone fruit species: apricot (*Prunus armeniaca* L.), cherry plum (*P. besseyi* × *P. hortulana*), Chinese bush cherry (*P. tomentosa* Thumb.), chokecherry (*P. virginiana* L.), Mongolian cherry (*P. fruticosa* Pallas), peach and nectarine (*P. persica* (L.) Batsch), plum (*P. domestica* L.), sand cherry (*P. besseyi* Bailey), sweet cherry (*P. avium* L.) and sour cherry (*P. cerasus* L.). The Genebank holds accessions of mostly Canadian origin (native, cultivars, selections and landraces). These are grown as trees in the open field (7-year-old orchards) and/or as potted plants under screen.

Field observations and sample collection

Field observations were carried out during spring 2002 and 2003, for symptoms associated with virus and virus-like diseases. A total of 645 trees (197 peach and nectarine, 183 sweet and sour cherries, 106 plum, 106 apricot, and 53 other cherry species) were sampled in May for ELISA testing. Tests were performed on young, fully-expanded leaves taken from the entire tree canopy in the field and from the main branches of potted plants. In November, old leaves with petioles were sampled from 336 selected trees (116 peach and nectarine, 84 sweet and sour cherries, 54 plum, 44 apricot, and 38 of other cherries) for tissue-printing hybridization.

ELISA

All trees were tested by DAS-ELISA (Clark and Adams, 1977) for the presence of PNRSV and PDV, and by DASI-ELISA (Cambra *et al*., 1994) for PPV. Serological reagents for PNRSV and PDV were commercial kits (Agdia, Elkart, Indiana, USA) and for PPV from (Durviz, Valencia, Spain).

Tissue printing and molecular hybridization

The presence of PLMVd and HSVd was checked by tissue-printing hybridization. Petioles of leaves were cut and printed on a nylon membrane in triplicate for each sample. The membranes were air-dried and submitted by mail to the analysis facility (Pallás *et al*., 2003). The digoxigenin-labelled riboprobes used for hybridisation were obtained by T7 RNA polymerase transcription of the linearized plasmids pHSVd and pPLMVd, described by Astruc *et al*. (1996) and Badenes and Llacer (2001) respectively. Pre-hybridisation and hybridisation were carried out at 68°C essentially as described by Pallás *et al*. (1998).

RT-PCR

RT-PCR was performed as described by Astruc *et al*. (1996) and Ambrós *et al*. (1998) to confirm all positive samples in the hybridization test.

Woody indexing on GF 305

Biological indexing was done for 297 accessions (one tree per accession) onto three seedlings of *P. persica* cv. GF 305. The indexed trees were selected from ELISA-negative material (where possible), in order to test for the presence of other graft-transmissible pathogens. Indicator plants were double chip budded with accession donors and maintained in a temperature-controlled greenhouse at 18–22°C for three-four months for symptom development.

Results

Field surveys

All trees and potted plants were routinely inspected during field and greenhouse surveys. In general, early symptoms of viruses and virus-like symptoms are easily confounded with mild mineral deficiencies and/or pesticide damage. Consequently it was difficult to identify virus and virus-like diseases by visual inspection and laboratory testing was essential.

ELISA

ELISA testing demonstrated that 131 (20.3%) of the tested trees were infected by at least one virus (Table 1). A total of 23 plums, 9 peaches and nectarines, 64 sweet and sour cherries, 22 apricots, and 13 other cherry species were found to be infected. The infection rates of the different species were: plum (21.6%), peach and nectarine (4.5%), sweet and sour cherry (34.9%), apricot (20.7%), and other cherry species (24.5%). PPV was not detected in any of the tested trees. The detected viruses were PNRSV and PDV, in single and mixed infections, both considered to be globally distributed (Diekmann and Putter, 1996). PNRSV was the prevailing virus, accounting for 74% of the infections in all tested trees, with the highest rate in apricot

Table 1. Viruses detected by ELISA in Clonal Genebank *Prunus* accessions (Harrow, Canada).

Species	No. of trees		Detected viruses			
	Tested	Infected	PPV	PNRSV	PDV	PDV+PNRSV
Peach and nectarine	197	9	0	4	5	0
Sweet and sour cherry	183	64	0	23	15	26
Plum	106	23	0	9	10	4
Apricot	106	22	0	19	2	1
Other cherry species	53	13	0	9	2	2
Total (20.3%)	645	131	0	64	34	33

Table 2. Viroids detected by molecular hybridisation in Clonal Genebank *Prunus* accessions (Harrow, Canada).

Species	No. of samples		Detected viroids	
	Tested	Infected	HSVd	PLMVd
Peach and nectarine	116	28	0	28
Sweet and sour cherry	84	0	0	0
Plum	54	0	0	0
Apricot	44	2	2	0
Other cherry species	38	0	0	0
Total	336	30	2	28

(90.9%). PDV was predominant in sweet and sour cherries, and plum, representing 64 and 60% of the infected trees respectively.

Molecular hybridisation and RT-PCR

Thirty stone fruit samples out of 336 were infected by viroids (Table 2). PLMVd occurred in 28 peach and nectarine samples (24.1%) of the following cultivars: Erlyvee, Harblaze, Hardired, Harko, Harbelle, Harken, Harland, Harrow Beauty, Harrow Rubirose, HW264, Redhaven, Silver Gold, Suncling, V68101, Vanity, Veeglo, Velvet, Vesper, Villa Doria and Vulcan. HSVd occurred in 2 apricot samples (4.5%) of the cultivars Bulida and Velkopavlovicka. These samples, determined to be positive by tissue-printing hybridization, were also positive by RT-PCR (data not shown).

HSVd has not been previously reported in North America (Singh *et al*., 2003) and this is the first report of its presence in the Canada. 'Bulida' is a Spanish cultivar which was previously shown to have a very high infection rate (75.3%) for HSVd in Southeastern Spain (Cañizares *et al*., 1998). In Canada (British Columbia) PLMVd was previously reported by Hadidi *et al*. (1997) and in Ontario by Torres *et al*. (2004). Our data on PLMVd incidence are consistent with those obtained previously by Torres *et al*. (2004), who tested a small number of trees of the same stone-fruit collection.

Four cases of mixed infections trees with viruses and viroids were found in nectarine: one in cv. Harblaze (PLMVd and PNRSV), two in cv. Hardired (PLMVd and PDV) and one in cv. Harko (PLMVd, PDV and PNRSV)

Indexing on GF 305

No viruses other than PDV were recovered from graft-transmission tests on GF305. PDV-infected plants were stunted showing a striking rosette of leaves. Only one plum accession induced abnormal proliferation of young shoots, small, leathery and pitted leaves. Work to identify the graft-transmissible pathogen related to these symptoms is ongoing.

Discussion

PPV was not detected in the collection, but other viruses and viroids, considered to be minor pathogens for the *Prunus* industry, were identified. In this initial study, 114 out of 297 accessions (38.4%)

Table 3. Sanitary status of Clonal Genebank *Prunus* accessions (Harrow, Canada) as determined by ELISA and molecular hybridization

Species	No. of accessions		Infection rate (%)
	Tested	Infected	
Peach and nectarine	81	26	32.1
Sweet and sour cherry	83	33	39.8
Plum	56	21	37.5
Apricot	41	19	46.3
Other cherry species	36	15	41.7
Total	297	114	38.4

were infected with at least one of these viruses and/or viroids (Table 3). The advent of sensitive and specific assay methods such as ELISA and molecular hybridization has provided the tools to accurately and efficiently detect viruses and viroids for which detection was previously difficult. Serological assays for viruses and molecular tests for viroids make it feasible to survey and monitor large numbers of specimens. They can also be used to enhance and maintain the sanitary status of collections such as the Clonal Genebank.

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