Occurrence of Nepoviruses in Small Fruits and Fruit Trees in Slovakia

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Abstract

A survey of nepoviruses in small fruits and fruit trees in thirteen localities of Slovakia was undertaken during the last three years. The samples of spontaneously infected small fruits and fruit trees with symptoms such as leaf yellowing, mottling, chlorotic spotting, vein clearing, chlorosis, dwarfing, and in some cases even plant dying, were analysed. Virus identification in collected samples from affected crops was performed by ELISA using polyclonal antibodies. The following quarantine nepoviruses were detected: *Tobacco ringspot nepovirus, Tomato ringspot nepovirus, Tomato black ring nepovirus, Raspberry ringspot nepovirus*, *Cherry leafroll nepovirus* and *Arabis mosaic nepovirus*. Screening of virus vector nematodes in the rhizosphere of infected plants was also undertaken. Three *Longidorus* (*L. elongatus, L. picenus* and *L. leptocephalus*) and four *Xiphinema* phytonematode species (*X. vuittenezi, X. diversicaudatum, X. taylori* and *X. pachtaicum*) were recorded in localities tested.

Keywords: nepovirus; phytonematode; nematode transmission of viruses

INTRODUCTION

Eight *Longidorus*, one *Paralongidorus*, and nine *Xiphinema* phytonematode species (Family *Longidoridae*, Order *Dorylaimida*) are vectors of the 12 out of 38 described nepoviruses, which cause diseases of economic importance (BROWN 2001). In 101 localities throughout Slovakia nematodes – virus vectors in the rhizosphere of fruit trees and soft fruits 13 species of ectoparasites of the family *Longidoridae* (genera *Longidorus, Paralongidorus* and *Xiphinema*) have been found (LIŠKOVÁ 1995). Nevertheless, information on the occurrence of nepoviruses are lacking. The presented study is aimed at the orchard surveys and the identification of nepoviruses that infect small fruits and fruit trees and at screening of the virus vector nematodes in the rhizosphere of infected plants.

MATERIALS AND METHODS

Plants were randomly collected from the orchards of Slovakia (at least five samples from each locality). The nematodes were extracted from the soil by using the decanting and sieving method of BROWN and BOAG (1988). The *Tomato ringspot nepovirus, Tomato black ring nepovirus, Raspberry ringspot nepovirus* and *Cherry leafroll nepovirus* quantity has been determined by double antibody sandwich enzyme-linked immunosorbent assay (ELISA) as described by CLARK and ADAMS (1977) by using an alkaline phosphatase conjugate and *p*-nitrophenyl phosphate as substrate. The used DAS-ELISA sets came from Loewe Biochemica GmbH (Germany) and from Bio-Rad Service Phytodiagnostics (France). *Tobacco ringspot nepovirus* (TRSV) and *Arabis mosaic nepovirus* (ArMV) were

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detected in plants by indirect ELISA as described by KOENIG and PAUL (1982) and by using Anti-mouse IgG alkaline phosphatase conjugate from Sigma. TRSV and ArMV polyclonal antisera were kindly granted by the Danish Government Institute of Seed Pathology for Developing Countries Antiserumbank (Denmark). The absorbance values at 405 nm were measured with a Dynatech 7000 microplate reader. The absorbance values greater than three times the average of the healthy control absorbance values were considered positive.

RESULTS

During the last three years three *Longidorus* and four *Xiphinema* phytonematode species were detected in the rhizosphere of fruit trees or small fruits collected from the thirteen localities of Slovakia listed in Table 1. Out of them only two virus-vector phytonematode species *Xiphinema diversicaudatum* and *Longidorus elongatus* were found according to the criteria described by BROWN (2001). By using ELISA the following quarantine nepoviruses were detected in plant samples: *Tobacco ringspot nepovirus, Tomato ringspot nepovirus, Tomato black ring nepovirus, Raspberry ringspot nepovirus, Cherry leafroll nepovirus* and *Arabis mosaic nepovirus* in investigated orchards (Table 2).

DISCUSSION

Significant occurrence of nematode-transmitted nepoviruses in naturally infected small fruits and fruit trees in Slovakia was detected. Results from these surveys have also revealed a distribution of the vector nematodes, from which mainly *Xiphinema* nematode species were considerably widespread. Especially *X. vuittenezi* were prevalent in small fruits and fruit trees. So far, *X. pachtaicum* and *X. taylori* were not described as virus vectors. A similar situation have *Longidorus* species – *L. picenus* and *L. leptocephalus* isolated from the rhizosphere of walnuts. The correlation of their occurrence with that of nepoviruses in these plants denote their possible role as virus vectors. Therefore our future research will be focused on tripartite interactions among nematode, virus and plant according to the criteria for assessing virus transmission by nematodes as proposed BROWN *et al*. (1995).

Table 1. Localities and occurrence of nematode species in principal crop hosts

Locality	Nematodes	Principal crop hosts
Bernolákovo	*X. vuittenezi*	strawberry, raspberry
Bošáca	*L. leptocephalus, L. picenus*	walnut
Čenkovce	*X. vuittenezi*	peach
Devín	*X. diversicaudatum, Longidorus* sp.	raspberry, strawberry, walnut, black current
Dvory nad Žitavou	*X. taylori, L. elongatus*	apple
	X. vuittenezi	plum
Ivanka pri Dunaji	*X. vuittenezi*	walnut, raspberry, grapevine
Kravany	*X. vuittenezi, X. pachtaicum*	peach
Mužla	*X. vuittenezi*	apricot
Prievoz	*X. vuittenezi*	raspberry, myrobalan
Pustá Ves	*L. leptocephalus, X. vuittenezi*	walnut
Svodín	*X. vuittenezi*	peach, plum
Topoľníky	*L. elongatus*	apple
Trhová Hradská	*L. elongatus*, *X. vuittenezi*	apple

Table 2. Nematode species, major crops affected and their associated viruses

Nematode species	Major crops affected	Viruses detected*					
		ToRSV	TBRV	RpRSV	ArMV	TRSV	CLRV
Longidorus spp.							
L. elongatus	apple	o	+	o	o	o	o
	raspberry	o	o	+	o	o	o
	strawberry	o	o	++	o	o	o
	black current	o	+	o	o	o	o
L. leptocephalus	walnut	o	++	+	o	o	+
L. picenus	walnut	o	++	+	o	o	+
Xiphinema spp.							
X. diversicaudatum	raspberry	+	o	o	o	o	o
	strawberry	o	o	++	+	+	o
	walnut	o	o	o	o	o	+
	black current	o	+	++	++	+	o
X. taylori	apple	o	+	o	++	++	o
X. vuittenezi	peach	o	+	++	o	o	o
	plum	o	++	o	++	++	o
	apple	o	+	o	+	+	o
	strawberry	+	o	++	++	++	o
	walnut	o	++	+	o	++	+
	raspberry	+	+	+	++	++	o
	grapevine	+	o	++	++	++	o
	myrobalan	+	o	o	o	++	o
	black current	o	+	++	o	o	o
	red current	o	o	++	o	o	o
	blackberry	o	++	++	++	++	o

*Response to ELISA:

++ moderate infection (A405 ≥ 0.50); + – traces of infection (A405 ≥ 0.15); o – not determined

ToRSV – *Tomato ringspot nepovirus*, TBRV – *Tomato black ring nepovirus*, RpRSV – *Raspberry ringspot nepovirus*, ArMV – *Arabis mosaic nepovirus*, TRSV – *Tobacco ringspot nepovirus*, CLRV – *Cherry leafroll nepovirus*

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