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SHORT NOTES

Incidence of *Prunus necrotic ringspot virus* in Jordan

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Summary. A survey of *Prunus necrotic ringspot virus* (PNRSV) incidence in Jordan stone-fruit growing areas was conducted during 2000–2002. A total of 2552 samples were collected from 72 commercial orchards, a mother block, 15 nurseries, and a varietal collection. A total of 208 almond, 451 apricot, 149 cherry, 250 nectarine, 1016 peach, and 478 plum trees were tested individually for PNRSV by the double-antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Around 15% of tested samples were infected with PNRSV. The virus incidence in almond, nectarine, plum, peach, cherry, and apricot was 24, 16, 16, 14, 13, and 10% of tested trees respectively. The level of viral infection was highest in the mother block (19%), and lowest in the samples from the nurseries (10%).

Key words: survey, PNRSV, DAS-ELISA.

Introduction

Stone fruits (almonds, apricots, cherries, nectarines, peaches, and plums) are important economic crops in Jordan. They are cultivated in almost all parts in the country but the type of cultivation varies with the area. Stone-fruit trees are traditionally grown in about 3837 ha in Jordan, peach being the most important and wide-spread species (1614 ha). It is followed by apricot, plum, almond, cherry, and nectarine grown on 784, 651, 479, 190, and 120 ha respectively (Anonymous, 2000).

The productivity of stone fruits in Jordan is falling behind that in many developed countries (FAO, 1998). This situation is attributed to agronomic, cultural, pathological, and entomological factors. The uncertain health status of planting material from the nursery plays a major part in the present deterioration of productivity (Taher, 1986).

Prunus necrotic ringspot virus (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) is pollen and seed-transmitted, which contributes to its spread in stone-fruit trees (Nemeth, 1986). The virus is also easily transmitted by common nursery grafting techniques. The practice of propagating cultivars on previously infected seedling rootstocks, has resulted in the widespread distribution of PNRSV as a result of the local and international trade in nursery material.

The present survey was conducted to estimate the incidence of PNRSV in stone-fruit orchards and to investigate how the incidence of the virus varies among major cultivars and among production areas.

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Materials and methods

Field surveys

Field inspections were carried out in the spring of 2000–2002 in the most important stone-fruit growing areas in Jordan. Samples were collected from a) commercial orchards throughout the traditional areas of cultivation, b) a mother block for bud-wood production established at Al-Hassan Station (Tafila) owned by Jordanian Ministry of Agriculture, c) nurseries of almond, apricot, cherry, nectarine, peach, and plum, and d) a varietal collection at the National Center for Agricultural Research and Transfer Technology (NCARTT) in Ash shawbak, Jordan.

Leaf samples were randomly collected each spring from symptomatic as well as from symptomless shoots. Samples for laboratory testing were examined visually for symptoms, then tested for PNRSV using biological and serological techniques. The incidence of PNRSV-infected trees was expressed as a percentage of all trees inspected.

ELISA test

The double-antibody sandwich assay (DAS-ELISA) was performed as described by Clark and Adams (1977), using commercially available IgG and alkaline phosphatase-conjugated PNRSV IgG (Bioreba AG, Reinach, Switzerland). ELISA readings were considered positive when the absorbance of sample wells was greater than at least twice the mean absorbance reading of two healthy control samples.

Mechanical transmission

During each survey, representative disease samples were biologically assayed on cucumber (*Cucumis sativus* cv. National Pickling). Inoculum was prepared by macerating the leaf tissues in 0.01 M phosphate buffer, pH 7.8, containing 0.001 M sodium-diethyldithiocarbamate and 2.5% nicotine in the presence of activated charcoal (100 mg ml^{-1}), using sterilized mortars and pestles. Plants were examined regularly for symptom expression. Inoculated leaves and shoot tips were then tested by DAS-ELISA.

Occurrence of PNRSV in other hosts

During the surveys, attempts were made to isolate PNRSV from some other fruit trees growing in and around stone-fruit orchards and nurseries. Leaf samples of apple, fig, grape, and pear trees showing virus-like symptoms were collected from Ajlun, Jarash, Madaba, Mafraq, Balqa, Ash shawbak, and Zarqa. Each sample was tested serologically for PNRSV with DAS-ELISA. Samples of rose with mosaic-type symptoms were obtained from commercial nurseries in Mafraq, Madaba, and Jarash. Rose-leaf tissue was mechanically inoculated to cucumber and tested for PNRSV using DAS-ELISA.

Results

Field surveys

A total of 72 commercial orchards, 15 stone fruit nurseries, a mother block, and a varietal collection were inspected during 2000–2002. Field symptoms were generally difficult to associate with PNRSV due to the poor growth of most orchards. When visible, symptoms appeared either on the entire tree canopy or only on a part of it. Symptoms varied considerably with the species, cultivar, location, and growing condition of the trees. Nevertheless, the observed incidence of symptoms and the degree to which symptoms were associated with PNRSV, as detected primarily by DAS-ELISA, and for some samples by mechanical transmission to cucumber, can be summarized as follows: in almond, PNRSV-infected trees exhibited severe leaf distortion, with a yellow, white, or light-green mosaic of a calico pattern. Some trees had varying proportions of their leaves with chlorotic blotches scattered at random over the leaf blades; in apricot, shot-hole symptoms on the leaves occurred in PNRSV-infected trees. PNRSV was detected in cherry trees showing tatter leaf and dark-green vein banding. In peach, a chlorotic oak-leaf pattern, followed by necrosis, small cankers on twigs and decline, was occasionally seen in PNRSV-infected trees. Severe bark necrosis with longitudinal splitting of the trunk and scaffold limbs were also associated with PNRSV infection. In plum, mild leaf mottling, leaf tattering, shot holes, chlorotic spots and lines, and oak-leaf pattern symptoms were observed on the leaves of some PNRSV-infected plants.

ELISA test

Survey results showed that, irrespective of *Prunus* species and location, more than 19% of all sam-

ples from the mother block were infected with PNRSV, while percent infection incidence of samples from commercial orchards, the varietal collection, and nurseries was 16, 13, and 10% respectively (Table 1).

Of the 2552 samples, 375 (15%) were infected with PNRSV. Infection rates ranged from 10% in apricot to 24% in almond, with intermediate infection in nectarine (16%), plum (16%), peach (14%), and cherry (13%) (Table 1).

The DAS-ELISA tests showed that PNRSV occurred in all regions surveyed, with an incidence ranging very widely among regions, from a high of 38% at Al-Mudawwara, followed by 30% at Ajlun, to a low of 3% at Irbid (Table 2).

Prunus necrotic ringspot virus was detected in

Table 1. Incidence of PNRSV in stone fruit trees/seedlings, surveyed in Jordan irrespective of location, in commercial orchards, nurseries, a mother block, and a varietal collection during 2000–2002 using DAS-ELISA.

Host plant	Source of tested samples				
	Commercial orchards	Nurseries	Mother block	Varietal collection	Total
Almond	17/75[a] (23)[b]	3/33(9)	30/100 (30)	-	50/208 (24)
Apricot	33/280 (12)	11/121 (9)	1/40 (3)	1/10 (10)	46/451 (10)
Cherry	15/82 (18)	4/67 (6)	-	-	19/149 (13)
Nectarine	16/78 (21)	3/82 (4)	-	21/90 (23)	40/250 (16)
Peach	96/612 (16)	13/103 (13)	10/73 (14)	23/228 (10)	142/1016 (14)
Plum	45/220 (20)	26/193 (13)	2/15 (13)	5/50 (10)	78/478 (16)
Total	222/1347 (16)	60/599 (10)	43/228 (19)	50/378 (13)	375/2552 (15)[c]

[a] No. of infected plants over No. of tested plants from different sources.
[b] Numbers in parentheses represent percentages.
[c] Overall average disease incidence on seedlings/trees in all regions in Jordan.
-, sample not collected.

Table 2. Incidence of PNRSV in stone fruit trees/seedlings from different locations in Jordan: commercial orchards, nurseries, a mother block, and a varietal collection, irrespective of *Prunus* species, surveyed during 2000–2002 using DAS-ELISA.

Region	Source of samples				
	Commercial orchards	Nurseries	Mother block	Varietal collection	Total
Irbid	1/31[a] (3)[b]	-	-	-	1/31 (3)
Ajlun	18/60 (30)	-	-	-	18/60 (30)
Jarash	17/119 (14)	9/186 (5)	-	-	26/305 (9)
Mafraq	47/321 (15)	-	-	-	47/321 (15)
Zarqa	25/140 (18)	-	-	-	25/140 (18)
Amman	2/12 (17)	-	-	-	2/12 (17)
Balqa	18/181 (10)	5/67 (8)	-	-	23/248 (9)
Jordan Valley	11/94 (12)	-	-	-	11/94 (12)
Madaba	21/199 (11)	45/317 (14)	-	-	66/516 (13)
Tafila	0/5 (0)	1/29 (3)	43/228 (19)	-	44/262 (17)
Ash shawbak	37/120 (31)	-	-	50/378 (13)	87/498 (17)
Al-Mudawwara	25/65 (38)	-	-	-	25/65 (38)

[a] No. of infected plants over No. of tested plants from each location.
[b] Numbers in parentheses represent percentages.
-, sample not collected.

one-year-old seedlings from all nurseries surveyed. Plum and peach scored the highest incidence of PNRSV-infected seedlings, at 13% (Table 1). Irrespective of species, the incidence of PNRSV infection was highest in Madaba (14%), followed by Balqa (8%), Jarash (5%), and Tafila (3%) (Table 2).

Of the almond cultivars surveyed in Al-Hassan Station, Ne Plus Ultra and S.F. 121, grown in a mother block and used for many years as budwood stocks for nurseries, were 100% infected. Of the cultivars Mestkawi and Tadmory tested in the mother block and the varietal collection, Tadmory was infected with PNRSV in the mother block, Mestkawi in both the collection and the mother block, while four cultivars (Ungarska, Peca nancy, Faranse, and Snare) from either the collection or the mother block were virus-free. Of 17 peach cultivars in the mother block and the varietal collection, only 5 were free of PNRSV: Dixired, Peento, Loring, Early Golddust, and J.H. Hale. None of the eight nectarine cultivars in the varietal collection was free of PNRSV. PNRSV occurred in two plum cultivars (Angeleno and Super Black) from the mother block and the varietal collection, but not in eight of the plum cultivars (Azaro, Black Star, Chili, Formosa, Golden Japan, Ozark Premier, Santa Rosa, and Stanley).

Mechanical transmission

Prunus necrotic ringspot virus produced numerous small, round, yellow rings on the cotyledons of *C. sativus* cv. National Pickling 3–5 days after inoculation. These symptoms soon became yellow spots, which coalesced to form a marked mottle. Within 24–48 h of symptom development on the cotyledons, yellow spots began to appear on the primary leaf, beginning at the base. Subsequently, yellow rings and mottle developed on the affected leaves. The apical growing point died, and the plants continued living for several weeks with only the two cotyledons. The results of mechanical transmission for positive ELISA samples were similar to ELISA tests.

Occurrence of PNRSV in other hosts

A total of 141 apple trees, 12 fig trees, 46 grape trees, 24 pear trees and 36 samples of rose with mosaic and broad chlorotic line or oak leaf symptoms were individually tested with DAS-ELISA. No PNRSV was isolated from any of the collected samples. Only one rose sample reacted with antiserum against PNRSV in DAS-ELISA.

Discussion

PNRSV was widespread in the regions surveyed, as indicated by the high incidence of infection in almond, apricot, cherry, nectarine, peach, and plum trees. PNRSV is pollen and seed-transmitted, and readily transmissible by grafting. These properties, in addition to the use of traditional cultural practices, and a lack of sanitary precautions and observance of regulations, have very probably contributed to the wide distribution of PNRSV in stone-fruit trees in Jordan. The results strongly suggest that infected scion buds, or rootstocks, or both, are being used in the propagation of nursery trees.

The relatively high mean infection level of 15% estimated by DAS-ELISA for the *Prunus* spp. widely grown in Jordan is not surprising since phytosanitary testing is not carried out, and uncontrolled propagating material circulates without restriction in the country. So far, there is no certification program ensuring virus-free or virus-tested vegetative propagative materials, nor have fruit-tree nurseries been subject to any regulation, so that nurseries can produce and sell budded or grafted trees without any controls.

The present survey, the largest in Jordan to date, provided a relatively clear picture of the phytosanitary status of stone-fruit species. The infection incidence of individual species appeared more satisfactory than that of other Mediterranean-stone fruit growing countries where comparable surveys have recently been conducted. The infection incidence in almond in Lebanon and Tunisia is 62 and 53%, respectively, compared with 24% in Jordan. In peach it is 14% in Jordan compared with 71% in Lebanon, 26% in Palestine, and 17% in Syria respectively (Jawher *et al.*, 1996; Zeramdini *et al.*, 1996; Al-Chaabi *et al.*, 2000; Jarrar *et al.*, 2001).

A major finding of the present study was the alarming infection incidence (up to 30%) of the almond mother block. Such high levels of infection in the almond mother block cv. Ne Plus Ultra and S.F.121, as well as in the varietal collection of nectarine (cv. Stark Red and Fantasia) shows

that there is a high risk of diseased nursery material being a primary cause of PNRSV. The mother block is a major source of budwood for propagation, and if prophylactic measures are not urgently adopted the phytosanitary status of almond and nectarine orchards established in the future will be at risk.

The survey underscores the urgent need for a certification program to prevent PNRSV introduction and spread. Such a program will have to include advisory work, the careful selection of healthy propagation material, and the compulsory eradication of infected trees. Only certified planting material or seedlings from approved organizations or institutions must be allowed to enter the country. The responsible agricultural regulatory authorities should draw up rules and regulations to stop the dissemination of PNRSV from nurseries.

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