THE REACTION OF *PRUNUS AVIUM* CLONE F12/1 PLANTS INOCULATED WITH PNRSV ISOLATES FROM DIFFERENT SPECIES OF *PRUNUS* AND ROSE PLANTS

E. Paduch-Cichal, K. Sala-Rejczak and J. Lewko

Abstract

The reaction of *Prunus avium* clone F12/1 to *Prunus necrotic ringspot virus* (PNRSV) isolates was determined. 12 PNRSV isolates from five *Prunus* species and three isolates from field-grown rose cultivars were investigated. Isolates PNRSV-PL7 or PNRSV-PL21 from plum trees cultivar ‘Empress’ did not infect *P. avium* clone F12/1. Plants of this clone were infected by isolates from almond, apricot, peach, plum (cvs. ‘Bluefre’, ‘Opal’), sour-sweet cherries and rose. Some differences in pathogenicity of 12 isolates to *P. avium* clone F12/1 were observed. The tested clone F12/1 can be a new host-plant for differentiating pathogenicity of PNRSV rose isolates.

**Key words:** *Prunus avium* clone F12/1, PNRSV isolates, *Prunus* species, rose plants

Introduction

The biological properties of *Prunus necrotic ringspot virus* (PNRSV) isolates were determined basing on reaction of some *Prunus* species. Symptoms appearing in the shock phase can be used to differentiate virus isolates. *Prunus avium*, *P. avium* clone F12/1, *P. serrulata* cv. ‘Shirofugen’, *P. persica* clone GF 305 and *P. persica* cv. ‘Elberta’ have been the best host plants to determine variation among PNRSV strains or isolates (Fulton 1970, Németh 1986). *Prunus serrulata* cv. ‘Shirofugen’ developed around the implante bud blackish-brown necrosis reaching xylem and gum two–three weeks after grafting with buds from sweet cherry, almond, apricot, plum and sour cherry trees infected with PNRSV. Clone GF 305 and cv. ‘Elberta’ of *P. persica* showed necrosis of shoot and leaves, mostly on the midribs on the lower surface after peach virus isolates inoculation. Some sweet cherry and sour cherry isolates caused on *P. persica* clone GF 305 or *P. persica* cv. ‘Elberta’ leaves chlorotic
rings or necrotic spots. Chlorosis, chlorotic rings, necrotic spots and shot-holes developed on these plants after almond, apricot and plum PNRSV isolates inoculation. The reaction of *P. avium* inoculated with sour- or sweet cherry virus isolates was similar, and additional enations are found mostly on the lower leaves surface. Peach PNRSV isolates caused on *P. avium* leaves necrotic spots and chlorotic rings.

Waterworth and Fulton (1964) demonstrated differences in intensity of typical shock symptoms on *P. cerasus* cv. ‘Montmorency’ and *P. pensylvannica* inoculated with 27 sour cherry PNRSV isolates. 17 out of these isolates produced abundant necrotic rings, shot-holes and leaf deformation. Other 10 isolates caused symptomless infection. Some of PNRSV isolates from different *Prunus* species induced on *P. persica* clone GF 305 mild leaf chlorosis or severe shot-holes or enations (Tuzovic and Digiaro 1992). Paduch-Cichal (2000) observed differences in pathogenicity of sour cherry PNRSV isolates on plants of *P. avium, P. avium* clone F12/1, *P. mahaleb* and *P. cerasifera*. Moury et al. (2000) described reaction of *P. persica* clone GF to different rose PNRSV isolates.

This paper reports the results of a study carried out to compare biological properties of different PNRSV isolates in *P. avium* clone F12/1.

**Materials and methods**

12 *Prunus necrotic ringspot virus* (PNRSV) isolates from different *Prunus* species and three virus isolates from field grown rose cultivars were used. The isolates were obtained from plants grown in Poland, Australia, Hungary and Italy (Table 1) and used to inoculate *P. avium* clone F12/1 plants. Plants were planted in the spring of 2004 into 10-litre pots filled with peat based medium. The inoculations were performed in greenhouse, in spring of 2004. Dormant buds from different *Prunus* species infected with PNRSV isolates were transferred to *P. avium* clone F12/1 using chip budding technique. Five plants of the clone were inoculated with each of the 12 isolates. Chip budding was performed at the height of 15 and 30 cm above level of the medium, using two buds from virus infected trees for each rootstock. Chip budding with dormant buds from healthy plants were considered controls. Place of budding was wrapped with budding strips to keep both components close to prevent of drying off. Wraps were removed 21 days after inoculation.

In summer of 2004, plants of *P. avium* clone F12/1 were inoculated with chips of bark of three PNRSV isolates from rose cultivars. Inoculation was done in greenhouse, as described above. Controls consisted of five chip budding with bark from each of healthy rose cultivar plants. All inoculated and control plants were grown in greenhouse for three months after inoculation, then moved to the field. In autumn, these plants were covered with peat to the height 15–20 cm and left over winter outside. In winter anti rodent protection was applied.

Inoculated plants of *P. avium* clone F12/1 were screened for virus symptoms 4, 12, 16, 24, 28 and 36 months after inoculation with PNRSV isolates from *Prunus* and 12 and 24 months after inoculation with PNRSV isolates from *Rosa*. 

The reaction of Prunus avium clone F12/1 plants inoculated with PNRSV isolates...

Table 1

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Source (host plant)</th>
<th>Origin (country)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNRSV-AL1</td>
<td>almond cv. ‘Strout’s Papershell’</td>
<td>Australia</td>
</tr>
<tr>
<td>PNRSV-AL17</td>
<td>almond cv. ‘Tardy non Pareil’</td>
<td>Italy</td>
</tr>
<tr>
<td>PNRSV-AprI/9</td>
<td>apricot cv. ‘Schönborn’ Wyk’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-MK</td>
<td>sour cherry unknown cultivar</td>
<td>Hungary</td>
</tr>
<tr>
<td>PNRSV-N2</td>
<td>sour cherry cv. ‘Lutowka’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-PE56</td>
<td>peach cv. ‘Meredith’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-PL1</td>
<td>plum cv. ‘Bluefre’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-PL7</td>
<td>plum cv. ‘Empress’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-PL9</td>
<td>plum cv. ‘Opal’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-PL21</td>
<td>plum cv. ‘Empress’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-PL38</td>
<td>plum unknown cultivar</td>
<td>Italy</td>
</tr>
<tr>
<td>PNRSV-R1</td>
<td>rose cv. ‘Queen Elizabeth’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-R2</td>
<td>rose cv. ‘Ingrid Bergman’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-R3</td>
<td>rose cv. ‘Montezuma’</td>
<td>Poland</td>
</tr>
<tr>
<td>PNRSV-SW2</td>
<td>sweet cherry cv. ‘Jabonlay’</td>
<td>Poland</td>
</tr>
</tbody>
</table>

All the inoculated plants were kept under observation throught the vegetation seasons 2004–2006, and regardless of whether or not they were showing symptoms, were individually tested using DAS-ELISA test according to Clark and Adams (1977) for the presence of PNRSV isolates. Control plants were also inspected visually and tested serologically.

Results

10 out of 12 PNRSV isolates were detected in all inoculated P. avium clone F12/1 plants by the serological DAS-ELISA test. Inoculation of these plants with virus isolates from plum ( cvs. ‘Bluefre’, ‘Opal’), sweet cherry, almond, sour cherry, apricot and peach was successful, but plants inoculated with PNRSV-PL7 or PNRSV-PL21 isolates were healthy.

During three years of observations differences in PNRSV isolates’ pathogenicity were observed and 10 virus isolates were divided into three groups dependent on symptoms intensity which developed on clone F12/1:

- group 1 – severe reaction: PNRSV-PL38, PNRSV-PE56, PNRSV-MK,
- group 2 – moderate reaction: PNRSV-AL17, PNRSV-N2, PNRSV-AprI/9, PNRSV-AL1,

Severe symptoms were showed after inoculation with isolates from group 1.
Severe leaf chlorosis, very abundant chlorotic rings/spots and severe shot-holes developed on plants inoculated with PNRSV-PL38 (in 2004). In the year of inoculation (2004) the isolate caused leaf chlorosis, very abundant chlorotic rings and necrotic spots. Very abundant chlorotic rings and necrotic spots were initiated in the second year after inoculation (2006; Phot. 1).

The reaction of \textit{P. avium} clone F12/1 inoculated with PNRSV-PE56 was similar in 2004 and 2005. Very abundant chlorotic rings with necrosis in the central part, chlorotic patterns, clear chlorotic patterns, severe shot-holes were noted. Symptoms developed in the second year after inoculation (2006) were weaker. Abundant chlorotic spots and rings, necrotic spots and weak shot-holes developed (Phot. 2).

\textit{Prunus avium} clone F12/1 reacted to PNRSV-MK isolate with very abundant chlorotic, necrotic spots, severe shot-holes and enations in 2004. (Phots. 3, 4). In the first year after inoculation (2005) symptoms were severe. All kinds of symptoms: chlorotic rings with necrotic rim, necrotic spots, necrotic rings between midribs and severe leaf shot-holes were very abundant. Leaf distortion, chlorotic patterns, abundant necrotic spots with brown-red rim, and weak shot-holes were noted on clone F12/1 inoculated with PNRSV-AL17 isolate. In the first year after inoculation (2005) symptoms were severe. All kinds of symptoms: chlorotic rings with necrotic rim, necrotic spots, necrotic rings between midribs and severe leaf shot-holes were very abundant. Leaf

![Phot. 1. Abundant chlorotic rings and spots on Prunus avium F12/1 leaf caused by PNRSV-PL38 (photo by K. Sala-Rejczak)](image)
distortion appeared (Phots. 5, 6). In the second year of inoculation the isolate caused only necrotic spots and chlorotic rings.

The reaction of clone F12/1 to PNRSV-N2 inoculation was severe in the first year after inoculation (2005). Chlorotic rings and necrotic spots were very abun-
dant and severe shot-holes appeared. In 2004 developed leaf chlorosis, abundant necrotic spots, weak shot-holes and enations. Single necrotic spots and weak shot-holes were observed in 2005.
During 2004-2006 abundant chlorotic spots, rings and necrotic spots were initiated by PNRSV-AprI/9 on *P. avium* clone F12/1 (Phot. 7).

PNRSV-AL1 isolate incited abundant necrotic spots and weak shot-holes on leaves during three years of study.
Phot. 8. Abundant chlorotic rings, necrotic spots and weak shot-holes on *Prunus avium* F12/1 leaf caused by PNRSV-SW2 (photo by K. Sala-Rejczak)

Phot. 9. Abundant chlorotic rings, necrotic spots and severe shot-holes on *Prunus avium* F12/1 leaves caused by PNRSV-R1 (two leaves on the right), few chlorotic rings and necrotic spots on *Prunus avium* F12/1 leaf caused by PNRSV-R3 (one leaf on the left) (photo by K. Sala-Rejczak)
The reaction of *P. avium* clone F12/1 plants to PNRSV-SW2 or PNRSV-PL9 or PNRSV-PL1 was weak (group 3).

The reaction of clone F12/1 to PNRSV-SW2 or PNRSV-PL9 isolates was quite similar. Abundant chlorotic rings, spots and patterns were noted in 2004. In the first year after inoculation (2005) severe leaf chlorosis, abundant necrotic spots, weak shot-holes, leaf distortion were noted (Phot. 8). In the second year after inoculation (2006) plants did not developed any symptoms.

*Prunus avium* clone F12/1 reacted to PNRSV-PL1 isolate with single necrotic rings, single chlorotic rings and necrotic spots during three years of study.

*Prunus avium* clone F12/1 inoculated with PNRSV-R1, PNRSV-R2 or PNRSV-R3, showed the first symptoms in May 2005. The reaction of plants to PNRSV-R1 isolate was severe, whereas to PNRSV-R2 was moderate and to PNRSV-R3 was weak. In the first and the second year after inoculation with PNRSV-R1 (2005 and 2006) plants developed abundant chlorotic rings, necrotic spots, severe leaf chlorosis and severe shot-holes (Phot. 9). Plants infected with PNRSV-R2 developed leaf chlorosis and single necrotic spots (2005), or weak leaf chlorosis (2006). Reaction of *P. avium* clone F12/1 was the weakest after PNRSV-R3 inoculation. Single chlorotic rings and/or single necrotic spots were reported.

**Discussion**

In Poland the first investigation on reaction of different *Prunus* species inoculated with sour cherry PNRSV isolates was described by Paduch-Cichal (2000). The experiments presented in this article revealed reaction of *P. avium* clone F12/1 inoculated with virus isolates from peach, sour-, sweet cherry, almond, apricot and plum trees.

According to Németh (1986) *P. avium* clone F12/1 is the best host plant to detect PNRSV. In our experiments the clone F12/1 plants were chip-budding inoculated with each of 12 PNRSV isolates of the different *Prunus* species. 10 out of 12 PNRSV isolates were detected in all inoculated plants by the serological DAS-ELISA test. Inoculation of these plants with virus isolates from plum (cvs. ‘Bluefire’, ‘Opal’), sweet cherry, almond, sour cherry, apricot and peach was successful, but plants inoculated with PNRSV-PL7 or PNRSV-PL21 isolates were healthy. Also Malinowski (personal communication) was not able to transmit PNRSV isolates from plum cultivar ‘Empress’ to *P. avium* clone F12/1.

The first PNRSV symptoms appear on this clone in the spring following inoculation. The shock symptoms developed on some branches. Chlorosis, chlorotic ringspot and severe necrosis spots developed on the leaves. Necrotic spots fall out very often and shot-holes are formed, giving the leaf a tattered appearance. The shock symptoms developed in majority of PNRSV infected trees in the year following inoculation, and in a smaller percent in the second or third year. The symptoms appearing after the shock symptoms were milder, fewer necrotic and mostly
chlorotic spots, rings or diffused yellow spots developed. Enations were found on the leaves near the margin between the veins, mostly on the 4th–6th leaf of young shoots, seldom on the same tree together with necrotic spots (Fulton 1970, Waterworth and Fulton 1964, Németh 1986, Paduch-Cichal 2000). In the experiments presented P. avium F12/1 plants exhibited similar symptomatological response to some of tested isolates from different Prunus species which had been described above.

According to observation of Moury et al. (2000) P. persica clone GF 305 is a proper plant to differentiate rose PNRSV isolates. The authors of this paper presented the reaction of P. avium clone F12/1 inoculated with three rose PNRSV isolates. They study revealed that plants were able to be a new host plant for differentiating virus rose isolates. This result is the first report in literature presenting symptomatological response of P. avium clone F12/1 to rose virus isolates.

Streszczenie

REAKCJA PRUNUS AVIUM KLON F12/1 NA INOKULACJĘ IZOLATAMI PNRSV Z RÓŻNYCH GATUNKÓW ROŚLIN RODZAJU PRUNUS ORAZ Z RÓŻ

Badano reakcję roślin Prunus avium klon F12/1 na inokulację przez okulizację 15 izolatami wirusa nekrotycznej pierścieniowej plamistości wiśni (Prunus necrotic ringspot virus, PNRSV). 12 izolatów wirusa otrzymano z różnych gatunków roślin rodzaju Prunus, trzy izolaty zaś pochodziły z róz gruntowych. W żadnej z inokulowanych roślin nie stwierdzono obecności izolatów PNRSV-PL7 ani PNRSV-PL21 otrzymanych z drzew śliw odmiany 'Empress'. Inokulacja roślin czereśni ptasiej klon F12/1 pozostałąmi izolatami wirusa otrzymanymi ze śliwy (odm. 'Bluefre', 'Opal'), czereśni, migdałowca, wiśni, moreli, brzoskwini i róż zakończyła się sukcesem. Wyniki badań pozwoliły na ustalenie różnic w patogeniczności pomiędzy izolatami PNRSV w stosunku do testowanych roślin. Wykazano, że czereśnia ptasia klon F12/1 to nowa roślina wskaźnikowa służąca do wykrywania różnic pomiędzy różnymi izolatami PNRSV.

Literature


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