

# SYMPTOMS ON APPLE AND PEAR INDICATORS AFTER BACK-TRANSMISSION FROM *NICOTIANA OCCIDENTALIS* CONFIRM THE IDENTITY OF APPLE STEM PITTING VIRUS WITH PEAR VEIN YELLOWS VIRUS

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## Abstract

Isolates of apple stem pitting virus (ASPV) from diseased apple trees were maintained in *Nicotiana occidentalis* then back-transmitted mechanically from the herbaceous host to apple seedlings and indexed by double budding on apple and pear indicators for the following syndromes: apple stem pitting, pear vein yellows, *Malus platycarpa* dwarf and quince sooty ringspot. Symptoms associated with stem pitting and *M. platycarpa* dwarf developed on Spy 227 and *M. platycarpa* apple indicators and symptoms associated with vein yellows on Jules d'Airolles, Le Curé and Nouveau Poiteau pear indicators. A number of indicators were indexed for symptoms on stems. Clear stem pitting symptoms were detected on Virginia Crab, Spy 227, R 2720, C 7/1 and *Pyronia veitchii*. These results confirm the identity of ASPV and pear vein yellows virus (PVYV) as one virus causing different syndromes in apple and pear. The isolates could be readily detected in *N. occidentalis* 37B by RT-PCR with ASPV-specific primers.

## 1. Introduction

Apple stem pitting is a latent virus of apple, occurring world-wide in commercial cultivars and frequently in combination with other latent viruses as apple chlorotic leaf spot virus (ACLSV) and apple stem grooving virus (ASGV). It has been reported to be related to pear vein yellows and other syndromes of apple and pear (Nemeth, 1986; Van der Meer, 1986). About a decade ago, isolates from stem pitting-infected apples and isolates from vein yellows- and stony pit-infected pears were successfully transmitted to *N. occidentalis* 37B and subsp. *obliqua* plants (Van der Meer, 1986), causing identical symptoms on these herbaceous hosts. Recently, major advances in the knowledge of particle and genome characteristics have been reported (Jelkmann, 1994) as well as serological and/or molecular biological evidence of close relationship between ASPV and pear vein yellows virus (PVYV) (Giunchedi and Poggi Pollini, 1992; Jelkmann, 1994; Koganezawa and Yanase, 1990; Pilotti *et al.*, 1995). However, for conclusive evidence that the same virus is the only causal agent of the above-mentioned syndromes a biological characterization by back-transmission from the herbaceous hosts to apple and pear is necessary. Recently, Leone *et al.* (1995) reported preliminary results on the mechanical transmission of the virus associated with ASP from *N. occidentalis* to apple seedlings and their indexing in the field on apple and pear indicators for ASP and PVY symptoms on leaves. This paper reports additional results obtained after following for a total of four years the woody indicators in the field, also for the occurrence of syndromes on their stems. Furthermore, detection of isolates of ASPV maintained in *N. occidentalis* 37B by RT-PCR is described.

## 2. Materials and methods

Isolates of ASPV obtained from infected apple trees and tested free from ACLSV and ASGV were maintained in *N. occidentalis* 37B or subsp. *obliqua* and back-transmitted to apple seedlings 'Golden Delicious' as described by Leone *et al.* (1995). These seedlings were indexed in a field experiment on apple and pear indicators by double budding as reported by Leone *et al.* (1995). Symptoms were assessed by visual inspections. For assessment of symptoms on stems, the indicators were cut below the union graft point, trunks were steamed for four hours, then placed in cold water and the bark was stripped off. The woody indicators used and the syndromes expected are shown in Tables 1 and 2.

Reverse transcription (RT)-PCR was performed on ASPV-infected *N. occidentalis* 37B leaves using the primer sets ASPF1-ASPR2 or ASPF1-ASPR5 and PCR conditions described by Malinowski *et al.* (this volume). Total RNA was extracted using an RNeasy kit (Quiagen).

## 3. Results and conclusions

We report some of the definitive results obtained in the field experiment set up by Leone *et al.* (1995) concerning the indexing on apple and pear indicators of apple seedlings wherein a virus associated with ASP and PVY disease was back-transmitted from *N. occidentalis*. The results of the assessment of symptoms on leaves and stems of woody indicators followed for a total of four years, are shown in Tables 3-5. From the results it appears evident that the same virus isolate is able to cause syndromes related to ASPV and PVYV in the respective apple and pear indicators. This conclusively confirms the identity of ASPV and PVYV as one virus causing different syndromes in apple and pear. These results are in accordance with the preliminary results presented by Leone *et al.* (1995) and with various claims of close relationship between ASPV and PVYV (Giunchedi and Poggi Pollini, 1992; Jelkmann, 1994; Pilotti *et al.*, 1995; Van der Meer, 1986; Yanase *et al.*, 1989). Differences in occurrence or intensity of symptoms on certain indicators were observed between the isolates. On leaves sometimes symptoms, once appeared, were lacking in succeeding years, indicating that the isolates were mild.

Detection of ASPV isolates Gloster 3 and VA 2-5.2 in *N. occidentalis* 37B by RT-PCR was possible using two primer sets developed by Malinowski *et al.* (this volume), directed against the polymerase open reading frame of ASPV (Fig. 1). Both isolates were back-transmitted to apple seedlings from *N. occidentalis* 37B and induced symptoms of PVYV in pear indicators.

In conclusions, a biological characterization of ASPV isolates, maintained in *N. occidentalis* plants and back-transmitted to apple seedlings, by indexing on apple and pear indicators in the field confirmed the identity of this virus and PVYV as one virus causing different syndromes in apple and pear. Two of these biologically characterized isolates can be easily detected in *N. occidentalis* 37B by RT-PCR with primer sets specific for the ASPV genome. The isolates are still being used in the field to ascertain whether ASPV or strains of this virus are also the cause of some fruit deformations in apple and pear (Nemeth, 1986).

## References

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Table 1 - Apple indicators for the field indexing of ASPV and syndromes expected

Indicator	Syndrome on leaf/stem
Virginia crab	stem pitting
Spy 227	epinasty, decline, stem pitting
<i>Malus platycarpa</i>	platycarpa dwarf
R 12740 7A	stem pitting at junction

Table 2 - Pear indicators for the field indexing of PVYV and syndromes expected

Indicator	Syndrome on leaf/stem
"Jules d'Airolles"	vein yellows
"Le Curé"	vein yellows
"Nouveau Poiteau"	vein yellows
C 7/1	sooty ringspot
<i>Pyronia veitchii</i>	decline, vein yellows, stem pitting

Table 3 - Assessment of symptoms on leaves of apple indicators for ASPV. (Number of infected/inoculated plants)

Isolate	Indicators			
	Virginia Crab	Spy 227	<i>M. platycarpa</i>	R 12740 7A
3049	no symptoms	epinasty (5/5)	plat. dwarf (5/5)	no symptoms
Gloster 1	no symptoms	epinasty (5/5)	no symptoms	no symptoms
Gloster 2	no symptoms	no symptoms	no symptoms	no symptoms
Gloster 3	no symptoms	epinasty (3/5)	plat. dwarf (5/5)	no symptoms
VA 2-5.1	no symptoms	epinasty (2/5)	no symptoms	no symptoms
VA 2-5.2	no symptoms	no symptoms	no symptoms	no symptoms
Pos. contr. <sup>1</sup>	no symptoms	decline (5/5)	no symptoms	no symptoms
Neg. contr. <sup>2</sup>	no symptoms	no symptoms	no symptoms	no symptoms

<sup>1</sup> Positive controls: inoculated with ASPV isolate 3049 or Gloster.

<sup>2</sup> Negative controls: mock-inoculated.

Table 4 - Assessment of symptoms on stems of apple indicators for ASPV. (Number of infected/inoculated plants)

Isolate	Indicators			
	Virginia Crab	Spy 227	<i>M. platycarpa</i>	R 12740 7A
3049	no symptoms	stem pitting	stunting (3/5)	junction pitting
Gloster 1	stem pitting	stem pitting	junction pitting	junction pitting
Gloster 2	stem pitting	stem pitting	junction pitting	junction pitting
Gloster 3	stem pitting	stem pitting	junction pitting	junction pitting
VA 2-5.1	stem pitting	stem pitting	junction pitting (2/5)	junction pitting
VA 2-5.2	stem pitting	stem pitting	junction pitting (3/5)	junction pitting
Pos. contr. <sup>1</sup>	junction pitting	stem pitting	junction pitting (1/5)	junction pitting
Neg. contr. <sup>2</sup>	no symptoms	no symptoms	no symptoms	no symptoms

<sup>1</sup> Positive controls: inoculated with ASPV isolate 3049 or Gloster.

<sup>2</sup> Negative controls: mock-inoculated.

Table 5 - Assessment of symptoms on leaves and stems of pear indicators for PVYV. (Number of infected/inoculated plants)

Isolate	Indicators				
	Jules d'Airolles	Le Curé	Nouveau Poiteau	C 7/1	<i>Pyronia veitchii</i>
3049	no sympt.	no sympt.	vein yellows	no sympt.	stem pitt. (4/5)
Gloster 1	no sympt.	vein yell. (3/5)	vein yellows	stem pitt. (4/5)	stem pitt. (2/5)
Gloster 2	vein yell. (2/5)	vein yell. (1/5)	vein yellows	stem pitt. (4/5)	stem pitting
Gloster 3	no sympt.	vein yellows	vein yellows	stem pitt. (2/5)	stem pitt. (4/5)
VA 2-5.1	vein yell. (2/5)	vein yell. (3/5)	vein yellows	stem pitt. (2/5)	stem pitt. (3/5)
VA 2-5.2	vein yell. (3/5)	vein yellows	vein yellows	stem pitting	stem pitt. (2/5)
Pos. contr. <sup>1</sup>	vein yell. (3/5)	vein yell. (5/5)	vein yellows	stem pitting	stem pitting
Neg. contr. <sup>2</sup>	no sympt.	no sympt.	no sympt.	no sympt.	no sympt.

<sup>1</sup> Positive control: inoculated with ASPV isolate 3049 or Gloster.

<sup>2</sup> Negative control: mock-inoculated.

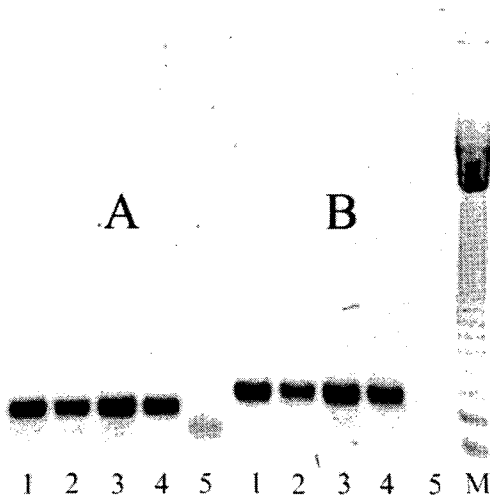


Figure 1 - Gel electrophoretic analysis of amplification products after RT-PCR of ASPV from infected *N. occidentalis* 37B leaves. A: primer set ASPF1-ASPR2 (fragment size: 202 nt); B: primer set ASPF1-ASPR5 (fragment size: 290 nt). 1: isolate Gloster 3, total RNA extraction; 2: as 1, diluted 1:100; 3: isolate VA 2-5.2, total RNA extraction; 4: as 3, diluted 1:100; 5: unrelated template (SMoV) in the reaction.