Increase of Plum Resistance to Natural Infections with *Plum pox virus*

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Abstract

The mechanical inoculation of the plum leaves of the trees in the nursery, in the first year of growth, has stimulated the activation of the plants' defensive system, fact that lead to their resistance to natural *Plum pox virus* (PPV) infections. The inoculum was prepared in buffer solution phosphate + Dieca of De Bistrita plum leaves infested by the PPV. Gas chromatography coupled with mass spectrometry (GC/MS) has shown significant differences in the quantity and quality composition of the volatile compounds in the treated and untreated plants, as well as in the infected trees.

Keywords: *Plum pox virus*; DAS-ELISA; gas chromatography; mass spectrometry

INTRODUCTION

The field resistance of plum to PPV may be characterized as a balance between the inoculum dose (virus concentration) and the plant's defensive system, controlled by specific genes. Stimulating the plant's defensive system, by mechanical inoculation with inoculum containing small concentrations of virus and proteins of the infected plant increases the plants' resistance to natural infections with virus.

MATERIALS AND METHOD

The young plum plants in the nursery (in their 1st year), of the De Bistrita and Stanley cultivars in the phases 7–23 leaves (May and June) were subject to mechanical inoculation with inoculum prepared from PPV infected De Bistrita leaves. The leaves were frozen and then mortared in the presence of a buffer solution of phosphate 1/15 M at pH 8 to which 0.3% DIECA was added. The ratio between the leaves' weight and the buffer solution was 1:3. After mortaring, the juice obtained was filtered through a gauze and part of it was concentrated with ammonium sulphate (40%), then centrifuged and dialyzed to phosphate buffer; the plants were then inoculated in the presence of carborundum. Two mechanical inoculations in the same plants were performed, at one month's distance between inoculations.

Visual observations and DAS-ELISA analyses were performed in the nursery two months after the last mechanical inoculation, and subsequently. In the fall the trees were taken out of the nursery and planted in the orchard in two different plots, one heavily infected with PPV and the other slightly infected.

For gas chromatography coupled with mass spectrometry CG/MS, the leaves were taken from the nursery in the month of August, being subject to steam distillation in order to separate the volatiles. The entrained volatile compounds were extracted with benzene, the benzenic layer was then dried on anhydrous magnesium sulphate, concentrated to 50 μl and analysed with the GL/MS Hewlett-Packard device. Chromatographic column: chapel type HP – 5, 30 m × 0.25 mm × 0.25 μm; chromatography program: injector 250°, column: 60°, 3 degrees/min at 200°C, isothermal at 200°C for 15 min; mass specter: 70 eV; data base: Wiley 275.

RESULTS AND DISCUSSIONS

The mechanical inoculation of the young plum trees with viral PPV inoculum has lead to the infection of half of the number of trees of the De Bistrita cultivar, in the year of inoculation, as shown by the DAS-ELISA test; this fact shows that in high viral concentrations the plant's defensive system is defeated. The number of trees infected is less than 100% in artificial infections by mechanical inoculation with concentrated viral inoculum; also less than 100% is the number of naturally infected trees (MINOIU *et al*. 2002); this fact also demonstrates an individual resistance of the plants.

The data in Table 1 show that the trees mechanically inoculated with non-concentrated viral inoculum were not naturally infected by PPV in the year of inoculation, even though they were planted in the heavily infected plot (plot 1). In comparison, the non-inoculated trees planted in the same plot got infected in the first year from planting. Starting with the second planting year, in plot 1, PPV infected trees were found in both variants, but in the treated trees the infection ratio was 25–60% below the control (untreated) – depending on the cultivar.

In plot 2 (slightly infected), infected trees start to come up beginning with the year 4, fact also confirmed by the DAS-ELISA test.

Budstick grafting maintains the resistance of the trees gained to PPV, while after chip budding the resistance decreases up to initial resistance.

The activation of the plum tree defensive system to PPV is also mentioned by HARTMAN (2002) in the case of hypersensitivity reaction.

Gas chromatography analysis, coupled with mass spectrometry show important differences between the variants (Figures 1–3). Thus, in the inoculated plants the absence of the product Phytol (Figure 1) is observed, the product is present in the non inoculated plants as well as in the PPV infected plants (Figures 2 and 3).

Table 1. The degree of infection in trees after mechanical inoculation with non-concentrated viral inoculum

Plum cultivar	Variants	Plum plot No. 1, heavily infected (100% infected trees)				Plum plot No. 2, weakly infected (< 2% infected trees)			
		infection ratio for each year							
		1 (1998)	2 (1999)	3 (2000)	4 (2001)	1 (1998)	2 (1999)	3 (2000)	4 (2001)
De Bistrita	Mechanical inoculation of plants	0/20	7/20	18/20	20/20	0/20	0/20	0/20	1/20
	Control (non-inoculated plants)	5/20	19/20	20/20	20/20	0/20	1/20	3/20	4/20
Stanley	Mechanical inoculation of plants	0/20	5/20	12/20	18/20	0/20	0/20	0/20	0/20
	Control (non-inoculated plants)	2/20	11/20	17/20	20/20	0/20	0/20	1/20	2/20

Remark: at the numerator – number of infected trees, and at the denominator the number of planted trees

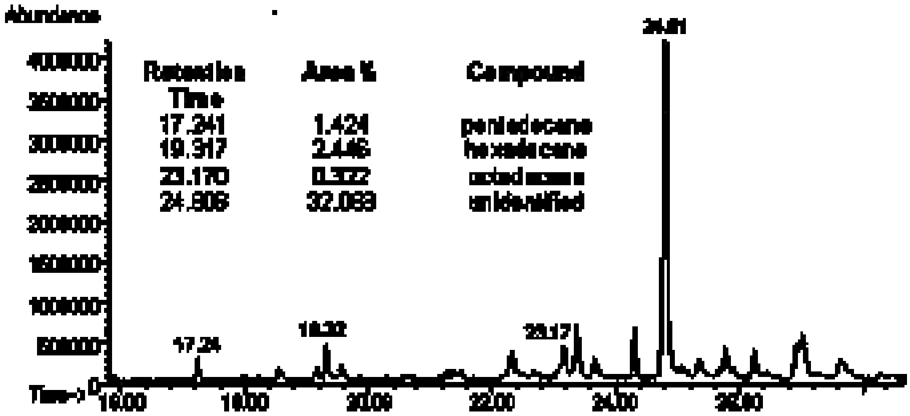

Figure 1. Gas-chromatogram of volatile compounds from plum mechanically inoculated with PPV leaves

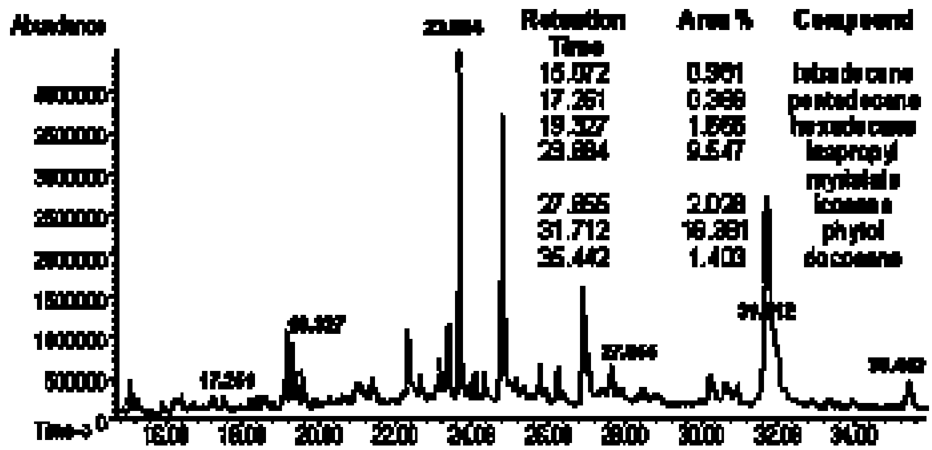

Figure 2. Gas-chromatogram of volatile compounds from healthy plum leaves

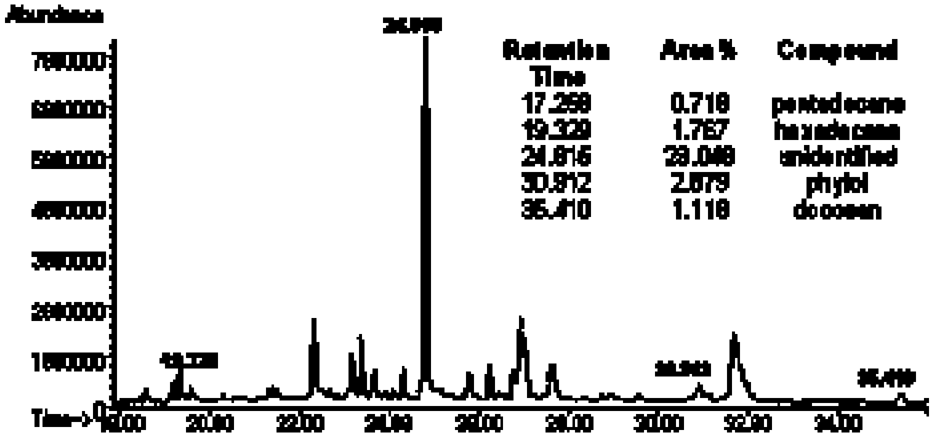

Figure 3. Gas-chromatogram of volatile compounds from PPV infected plum leaves

Isopropyl mystrat (Figure 3) was for the first time found in the PPV infected trees, substance that is missing in the healthy trees, treated and not treated, and could probably be a new diagnose test for the *Plum pox virus*.

The superior alcans identified probably come from the treatments with certain pesticides (paraffin oils).

Conclusions

Mechanical inoculation of the plum leaves with PPV inoculum has lead to the increase of tree resistance to natural infections with this virus and to the modification, within certain boundaries, of the volatiles composition. For the first time isopropyl mystrat compound was found in the PPV infected trees.

References

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