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Effects of economically important virus diseases on the expression of some pomological traits and nutritional compounds in GM plum cultivar HoneySweet (*Prunus domestica L.*)

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Abstract

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Economically important viruses infect plums and other stone fruits cause lower yields, fruit size and quality and also affect its chemical composition. Fruits of the genetically modified (GM) plum 'HoneySweet' growing on trees deliberately infected with *Plum pox virus* (PPV), *Prune dwarf virus* (PDV) and *Apple chlorotic leaf-spot virus* (ACLSV) were analysed in detail to determine what effects these virus infections have on selected pomological traits, dissolved solids and titratable acids. Assessments of the fruits were made in the years from 2011 to 2014. The GM plum 'HoneySweet' was chosen for this experiment, and uninfected trees of the same variety in the same orchard were used as control. It was shown that there was no effect on dissolved solids resulting from PPV infections, either alone or in combination with the other viruses, but that there was a significant effect on levels of titratable acids where trees had been inoculated with all three viruses (treatment I). Regarding pomological traits, in most cases there were no significant effects seen to affect internal characteristics, only that in the assessments were lower although still of an acceptable quality for commercial fruit.

Keywords: inoculation; *Plum pox virus*; *Prune dwarf virus*; *Apple chlorotic leaf-spot virus*; pomological traits; nutritional compounds

Plum pox virus (PPV) has become the main factor limiting the establishment of new fruit orchards. In the Czech Republic, it affects mainly peaches and plums, but in a range of other European countries it is also a big problem for apricots, especially in those orchards planted with the newer and much more susceptible varieties. The spread of PPV can only be limited by reducing the sources of infection and using virus-free material when planting new orchards. It is also essential to observe the eradication guidelines, especially in areas in which the virus is not widespread, when destroying infected trees. If preventive measures against aphid vectors are also taken, then it should be possible to keep the trees in newly planted fruit orchards free of PPV infections for at least 10 years.

The best chances for success in establishing new fruit orchards therefore lies with plums, since most of the commercially desirable varieties are tolerant to PPV and so, even when infected, serious financial losses are unlikely. In the future there will also be PPV-resistant varieties available, mostly trans-

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genic, and these are currently undergoing trials in the EU, including the Czech Republic.

Very little has been published about the possible changes in the chemical make-up of fruits grown on trees infected by virus diseases. Polák et al. (2012) reported that fruits of the genetically modified (GM) 'HoneySweet', after joint infections of PPV with *Plum dwarf virus* (PDV) and/or *Apple chlorotic leaf-spot virus* (ACLSV), appeared to suffer no deterioration in either quality or quantity. Useniket al. (2014) measured the levels of individual sugars and other compounds in the plum 'Brkinska češpa' (*P. domestica* L.), in fruits produced by trees suffering from short-term and long-term PPV infections, and determined that the fruits from long-term infected trees had the poorest pomological traits and also the most modified composition regarding nutritional and phenolic compounds Strick and Martin (2003) measured the effect of the Raspberry bushy dwarf virus on yield, fruit quality and growth of canes in the blackberry 'Marion'. They found that there was no effect on cane growth or fruit number, but there were reductions in yield (40–50%), fruit weight (23–40%), and drupelet number per fruit (36–39%) when compared with uninfected plants.

The aim of this work was to determine how the *Plum pox virus*, alone and in combination with the viruses responsible for "pseudo plum pox", affects various pomological traits and certain selected nutritional compounds in the fruits of the GM 'HoneySweet'.

MATERIAL AND METHODS

The GM plum 'HoneySweet' was deliberately infected with PPV virus in combination with two other viruses, ACLSV and PDV. The combinations were: I – PPV, PDV and ACLSV, II – PPV and PDV, III – PPV and ACLSV, IV – PPV on its own, and V – represents a Control group of virus-free trees, growing at an isolated area at the Václav Havel International Airport in Prague.

The trees were planted in 2003 on a clonal rootstock declared by nursery Fytos Plzeň as virus-free rootstock named St. Julien, with a spacing of 2.6 × 3.0 m, and were subsequently pruned to achieve a free-growing open crown. Each year, there was winter and summer pruning, application of mineral fertilizers, mechanical cultivations between the rows and weeding around the base of the trees before the orchard was finally grassed down, after which the following operations took place as required: grass-cutting, irrigation in dry periods, protection against damage by wildlife (including field voles), spraying against aphids (Pirimor and Chess, for example) and other insect pests, and during flowering two applications against Plum sawfly *Hoplocampa minuta* (Christ) (Calypso, Reldan) and three against *Monilinia* spp. (Horizon, Teldor, Rovral) with a further single application again before the start of fruit ripening.

Growing conditions in Prague are classified as being suitable for plum production, at 370 m above the sea level. The average annual temperature in the period from 1961 to 1990 was 7.9°C overall, but 14.0°C from March to September. Average humidity was 77.3%, average annual precipitation was 525.9 mm and average annual sunshine was 1,668 hours.

The quality of the fruits was assessed on the basis of internal and external parameters in accordance with methods described in the international UPOV Species Code: PRUNU_DOM (adopted 6.11.03) and also with modifications of the Czech standards for evaluating apricots (Vachůn et al. 1991).

The quality of HoneySweet fruits (variants I–V) was compared with quality of plums, 'Stanley', and 'Jojo':

(a) External fruit parameters assessed: uniformity of shape (1–9 points), appearance (1–9 points), weight (g), height, width and depth (mm), skin thickness (mm.), shape (1–9 points), ground skin colour (1–9 points), over skin colour (1–9 points), fruit cracking(1–9 points) and flesh firmness (1–9 points).

(b) Internal fruit parameters assessed: flesh colour (1–9 points), flavour (1–9 points), ease of peeling (1–9 points), % of solids measured by refractometer, stone weight (g), stone proportion of overall weight (%) and kernel flavour (bitter, slightly bitter or sweet).

One to two days after harvest 30 fruits for each treatment were assessed, and the data were analysed statistically using the software programmes Statistica 9 (StatSoft, Inc, Tulsa, USA). Fruit uniformity: an assessment of 1 on the scale means completely uneven (for shape and colour), 9 means completely even. Attractiveness: 1 – unattractive, 9 – very attractive. Shape: 1 – elongated, 4 – round, 6 – oval, 9 – inverted egg-shape. Ground skin colour was graded as follows: 1 – green, 2 – yellow-green, 3 – yellow, 4 – orange-yellow, 5 – purple/violet, 6 –violet blue, 7 to 9 – dark blue. Flesh colour was

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graded: 1–2 –whitish, 3–4 – yellow-green, 5–6 – yellow, 7–8 – orange, 9 – red. Flesh firmness is ranked 1 – soft, 9 – very firm. Flavour: 1 – extremely bad, 3 – bad, 5 – acceptable, 7 – good and 9 – excellent.

Total titratable acids were measured for each treatment (g/kg).

Dissolved solids were measured using an Abbey refractometer (Model No. 2WAJ, Desktop, range 0~95%, Guangdong, China) and expressed in Brix degrees.

A similar set of observations were made at the same time on two standard or commercial varieties of plums cvs 'Stanley' and 'Jojo' to provide a basis for comparison, but could not be used directly in the sense of controls since they were grown under different conditions in a different climatic region in Horticulture faculty in Lednice.

The data were statistically analysed using single-factor dispersion analysis and Scheffe's test from programmes provided by StatSoft (Statistica 10, StatSoft, Inc, Tulsa, USA).

RESULTS AND DISCUSSION

Subjective assessments of external and internal fruit characteristics

In general, it can be concluded that there were no significant differences between the four treatments

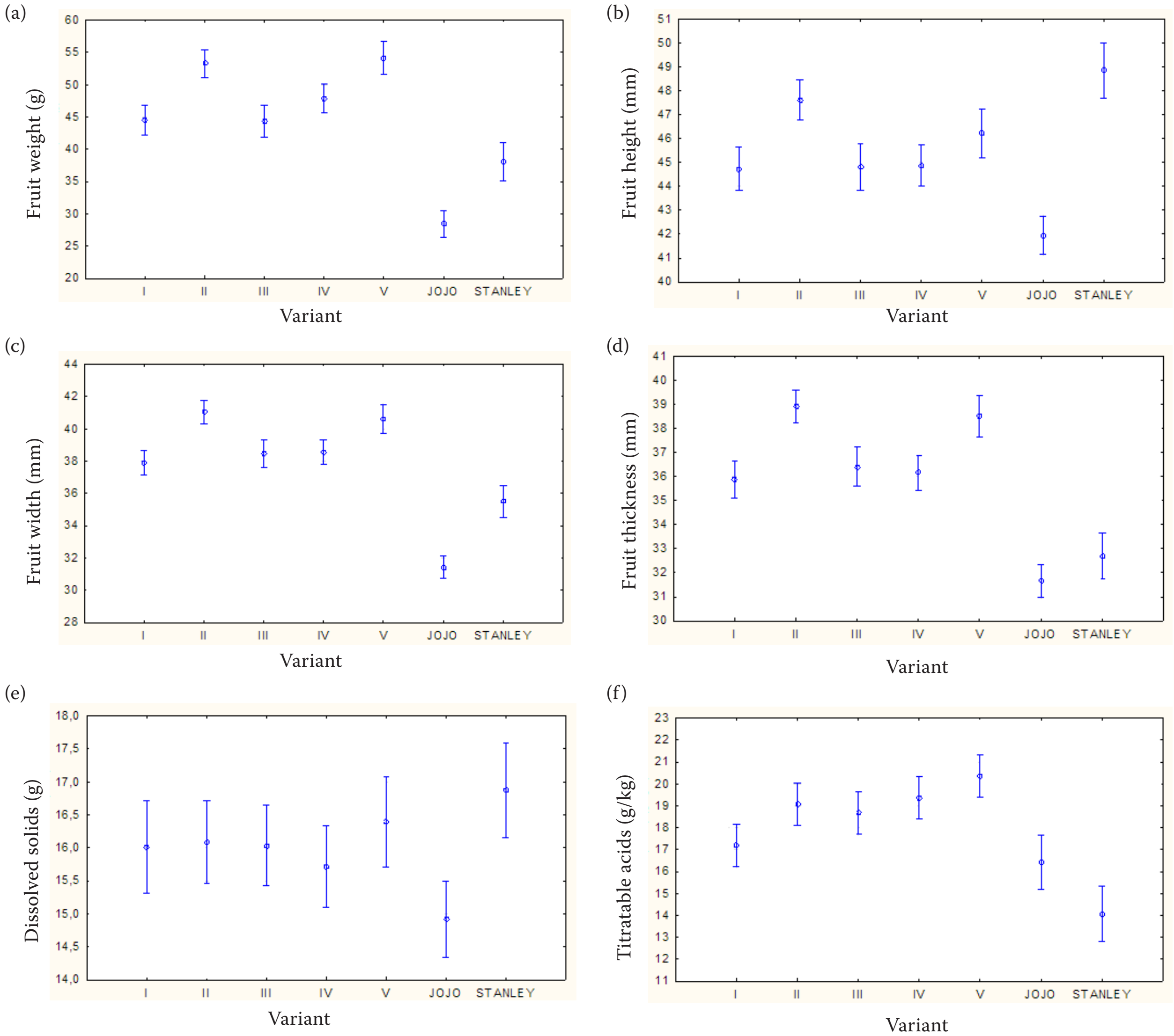

Fig. 1. Statistical analysis of fruit (a) weights, (b) heights, (c) width, (d) thicknes, (d) dissolved solids and (f) total titratable acids

vertical lines indicate the 95% confidence limits

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and the controls with regard to basic skin colour, flesh colour and fruit-splitting.

Uniformity and attractiveness of fruits in all treatments was very good and rated 8, except for treatments II and IV which were rated 7 overall. Attractiveness was rated 8 in the control and treatment I, but rated 7 in the other treatments on average. The treatments had no great effect in changing fruit shape, but treatments I and III were rated 7 (oval to inverted egg-shape), while other treatments including the controls were rated 6 (oval). Skin colour was practically the same in all treatments and rated 8 (very intensive), the exception being treatment III which was rated 7.

Flesh firmness was only assessed subjectively and on average it was rated 6 (medium firm) in the controls and treatments II and III, and rated 5 (average firmness) in treatments I and IV. Flavour, one of the most important attributes, was rated good with 7 points except in treatment IV which was rated 6, i.e. average to good. The bitter flavour of the kernels was not different from the control in either of the treatments.

No statistical differences between treatments were shown in either of the above characteristics.

In conclusion, since none of the treatments produced fruits significantly different to the controls, it can be said that for these subjectively assessed attributes the presence of these virus infections is of no great consequence for fruit growing, from either a horticultural or commercial perspective.

Objective measures of external and internal fruit characteristics

Fruit weight is a very important commercial attribute. The highest average weights during the

Table 1. Average values of pomological traits (2011–2014)

Parameter	Variant						
	I	II	III	IV	V	'Stanley'	'Jojo'
Uniformity of fruits (1–9)	8	7	8	7	8	8	8
Attractiveness (1–9)	8	7	7	7	8	7	7
Fruit weight (g)	44.47	53.29	44.36	47.86	54.16	38.10	28.40
Fruit height (mm)	44.74	47.63	44.80	44.87	46.23	48.96	41.95
Fruit width (mm)	37.90	41.05	38.47	38.57	40.63	35.50	31.43
Fruit thickness (mm)	35.88	38.92	36.41	36.17	38.50	32.70	31.66
Flesh thickness (mm)	11.72	12.91	11.37	11.64	11.57	7.47	7.63
Fruit shape (1–7)	7	6	7	6	6	7	6
Basic fruit colour (1–9)	7	7	7	7	7	7	8
Over skin colour (1–7)	8	8	7	8	8	9	7
Fruit splitting (1–9)	9	9	9	9	9	9	9
Flesh colour (1–9)	5	5	5	5	5	5	6
Fruit firmness (1–9)	5	6	6	5	6	8	8
Flavour (1–9)	7	7	7	6	7	7	7
Ease of peeling (1–9)	7	7	7	7	7	8	7
Dissolved solids (°Brix)	16.02	16.08	16.04	15.72	16.40	16.87	14.92
Stone weight (g)	2.07	2.18	1.87	2.01	2.19	2.20	1.70
Stone weight compared to overall fruit weight (%)	4.42	4.03	4.40	4.10	4.11	5.83	6.02
Stone height (mm)	24.43	24.69	23.64	24.30	25.43	27.62	25.33
Stone width (mm)	13.60	14.26	13.54	13.60	14.30	13.24	13.48
Stone thickness (mm)	7.19	7.69	7.13	6.94	7.29	7.02	5.90
Kernel flavour (B – bitter)	B	B	B	B	B	B	B
Titratable acids (g/kg)	17.20	19.08	18.70	19.38	20.35	14.07	16.42

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same cropping period were shown by the control (54.1 g) and treatment II (53.2 g), although these were not statistically different from the others (Fig. 1a). Fruit heights were also statistically indistinguishable, but again the control and treatment II were greatest (Fig. 1b). Fruit widths were also greatest in the controls and treatment II, but here at least treatment I was shown to have significantly narrower fruits (37.1 mm) (Fig. 1c). Thickness of fruits was greatest in treatment II (38.9 mm) and the controls (38.5 mm), and these were significantly different from treatments I and IV (but not treatment III) (Fig. 1d). The range of values for dissolved solids as measured by a refractometer were not significantly different in any of the treatments, ranging from 15.72°Brix for treatment IV to 16.40°Brix for the control (Fig. 1e). Total titratable acids ranged from 17.2 g/kg in treatment I to 20.35 g/kg in the control, with only these two extreme values being statistically significantly different (Fig. 1f).

Average values for objectively and subjectively assessed characteristics for the whole period of observations are summarized in Table 1.

Lius et al. (1997) described a transgenic clone of papaya ('Sunset', R0 clone 55-1) and compared fruit quality and growth with a non-transgenic clone after inoculation with papaya ringspot virus. Fruit brix, morphology and fertility of the 55-1 clone plants were all normal, and no pleiotropic effects of the coat protein gene were observed. The absence of any obvious "ill-effects" arising from the inoculation of the virus is a similar result to ours following inoculation of the GM plum 'HoneySweet' with a range of virus infections.

The conclusion is that fruits from treatments I, III and IV had lower values for fruit weight; while for treatment II there were no significant differences for either fruit weight or height. Treatment I had lower values for all observed characteristics, showing that the combined effect of three simultaneous virus infections had the greatest effect.

CONCLUSION

The aim of this work was to establish which effects the *Plum pox virus* has, alone and in combination with another two viruses which cause pseudopox, on the development of certain pomological traits and nutritional compounds in fruits of the GM plum 'HoneySweet'.

It was shown that the infections had no significant effect on measurements of dissolved solids, nor on total titratable acids (with the exception of treatment I). Differences in pomological traits were greater, as fruit weight, width and thickness were lowest for the mixture of viruses (treatment I) and PPV alone (treatment IV).

The subjectively assessed internal and external pomological traits were either not different between treatments or of no commercial significance. The GM plum 'HoneySweet' was shown to have a high level of resistance not only to PPV but also to other two commercially important viruses. The effect of artificial virus infections induced by using infected grafting material on fruit quality was minimal and in the majority of cases it was not statistically significant. The quality of 'HoneySweet' fruits was better than the quality of fruits, 'Stanley' and 'Jojo' except fruit firmness.

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