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Reaction of transgenic plum cv. HoneySweet to the *Plum pox virus* after a severe infection of *Monilinia* sp. – Short communication

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Abstract: Resistance to *Plum pox virus* (PPV) in transgenic *Prunus domestica* L., clone C5 (cv. HoneySweet) was evaluated in a regulated field in the Czech Republic for fifteen years (2002–2016). PPV mild symptoms appeared in C5 trees only in several leaves situated close to the point of inoculum grafting up to 2010. No symptoms of PPV were observed in the years 2011–2013 and results of ELISA and RT-PCR detection tests were negative. In the twelfth year (2013), there was a severe unusual natural attack of plum trees by *Monilinia* sp. This *Monilinia* sp. attack occurred only one time – in 2013. There was no *Monilinia* sp. infection in 2002–2012 and in 2014–2016. Mild PPV symptoms reappeared in several leaves of transgenic plum trees in the next two years (2014–2015) and the presence of PPV was proved by DAS-ELISA and confirmed by RT-PCR.

Keywords: interaction; sharka; monilia; plum; genetic modification

Effective host plant resistance to *Plum pox virus* (PPV) in plums was developed by Scorza *et al.* (1994). The genetically modified (GM) plum clone C5 (cv. HoneySweet) was deregulated in the USA in 2012.

An experimental orchard of the plum *P. domestica*, cv. HoneySweet was established in the Crop Research Institute, Prague-Ruzyně (Czech Republic) in 2002 (Polák *et al*. 2005). Details were presented in a previous study (Polák *et al*. 2017). The influence of *Prune dwarf virus* (PDV) and *Apple chlorotic leaf-spot virus* (ACLSV) in a mixed infection (PPV+PDV, PPV+ACLSV, PPV+PDV+ACLSV) was also studied.

Preliminary and partial results obtained up to 2013 were published (Polák *et al.* 2005, 2012, 2017). A severe unusual natural attack of plums by *Monilinia* sp. appeared in 2013. Results of the investigation in 2014–2016 after this attack are presented.

MATERIAL AND METHODS

Plants of plums infected with PPV, PPV+PDV, PPV+ACLSV, and PPV+PDV+ACLSV were investigated in the years 2003–2016. Plants were monitored by symptom evaluation twice a year during vegetation period – at the end of May to mid-June and at the mid-August to the beginning of September. ELISA and RT-PCR (Reverse transcription PCR) tests were performed every year in June. Leaves of all trees were tested by DAS-ELISA ELISA (Double Antibody Sandwich ELISA) and approximately one third of all trees were also tested by RT-PCR.

The unusually severe natural infection by *Monilinia* sp. appeared after the cold and rainy weather during the full bloom phenophase in 2013. The only one spray with Signum fungicide could be realised at the

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beginning of flowering. Plum trees cv. Jojo growing close to the GM plum cv. HoneySweet showed the same severe infection by *Monilinia* sp. There was no difference in the susceptibility to the *Monilinia* sp. infection between transgenic plum cv. HoneySweet and non-transgenic control plum cv. Jojo.

RESULTS

The influence of Monilinia sp. attack on PPV infection. Severe natural *Monilinia* sp. attack (Figure 1) resulted in dying of whole branches of trees, which were then cut down, leading to weakening of vigour of the trees. Moreover, mild or very mild PPV symptoms – diffuse spots and rings – appeared in some basal leaves of some HoneySweet trees in 2014 and 2015. The presence of PPV was confirmed both by ELISA and RT-PCR. No PPV symptoms were observed in leaves of plum, cv. HoneySweet trees inoculated with PPV-Rec (PPV-recombinant strain) and the other three combinations during 2016, but low presence of PPV was confirmed by ELISA in leaves of three trees infected with PPV-Rec+PDV+ACLSV, in one tree infected with PPV-Rec+PDV, in two trees infected with PPV-Rec+ACLSV, and in one tree infected with PPV-Rec. Results of PPV detection by DAS-ELISA during 2013–2016 in cv. HoneySweet trees inoculated with PPV-Rec, and different combinations with PDV and ACLSV are presented in Table 1, where rapid increase of positive reactions in 2014 and slow decrease of a number of positive reactions in next years is clearly demonstrated. The presence of PPV was confirmed by ELISA and RT-PCR not only in symptomatic leaves, but also in several fruits (ca. 5%) showing no symptoms.

Table 1. PPV detection by DAS-ELISA during 2013–2016 in cv. HoneySweet plants inoculated with PPV-Rec, PDV, and ACLSV in different combinations

Year	PPV-Rec positive plants/			
	PPV-Rec inoculated plants	PPV+PDV inoculated plants	PPV+ACLSV inoculated plants	PPV+PDV + ACLSV inoculated plants
2013	0/9	0/10	0/11	0/11
2014	7/9	5/10	2/11	6/11
2015	5/9	3/10	3/11	4/11
2016	1/9	1/10	2/11	3/11

PPV – *Plum pox virus*; DAS-ELISA – Double Antibody Sandwich ELISA; PPV-Rec – *Plum pox virus* – recombinant strain; PDV – *Prune swarf virus*; ACLSV – *Apple chlorotic leafspot virus*

Figure 1. Tree (**A**) and detail (**B**) of cv. HoneySweet severely infected with *Monilinia* sp.

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Symptoms and detection of PDV and ACLSV in transgenic plum, cv. HoneySweet during 2003–2016. No symptoms of PDV appeared during the vegetative periods of 2003–2016. PDV was not detected by ELISA in transgenic parts of trees inoculated with PPV-Rec+PDV and PPV-Rec+PDV+ACLSV. No symptoms of ACLSV appeared during the vegetative periods of 2003–2016, however, ACLSV was detected by ELISA and RT-PCR in leaves of transgenic parts of trees inoculated with PPV-Rec+ACLSV and PPV-Rec+PDV+ACLSV every year. No influence of PDV and ACLSV on PPV infection in transgenic plum, cv. HoneySweet was demonstrated during 2003–2016.

DISCUSSION

Preliminary and partial results of the investigation up to 2012 were published (Polák *et al*. 2005, 2012). Resistance in cv. HoneySweet plums evaluated for PPV, and combinations of PPV with *Prune dwarf virus* (PDV), and *Apple chlorotic leaf spot virus* (ACLSV) was demonstrated in a regulated field trial in the Czech Republic in 2002–2013 and published (Polák *et al*. 2017).

Fifteen years of field tests in the Czech Republic proved not only the presence of very mild PPV infection after grafting, but also the active elimination of PPV in trees during 10 years and the elimination of PPV again after the severe natural *Monilinia* sp. attack. To our knowledge, it is the first presentation that fungal pathogen can influence viral pathogenesis.

Minimal PPV symptoms observed in graft-inoculated trees disappeared during the following three years, and no PPV leaf symptoms and positive ELISA tests were found after the sixteen-year field trial. The stability of resistance in plum, cv. HoneySweet in the field conditions was affected by the severe natural *Monilinia* sp. infection, but trees recovered again during three years (2014–2016) after the *Monilinia* sp. attack. The sharka disease symptoms development and their subsequent disappearance over the time in plum, cv. HoneySweet were first observed in our field experiment. European field tests clearly demonstrated the resistance of plum, cv. HoneySweet to PPV infection through aphid vectors and by graft inoculation (for review see Scorza *et al.* 2013).

We have shown the high quality of plum, cv. HoneySweet fruits harvested both from non-graft-inoculated trees and trees under the high and permanent infection pressure of PPV and combinations with PDV and ACLSV (Sochor *et al*. 2015). The durable resistance of cv. HoneySweet to several PPV strains (Ravelonandro *et al.* 2001) and high fruit quality hence make the HoneySweet plum cultivar among the plum growers and owners of fruit tree nurseries a tool for effective control against PPV.

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