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Gene flow was not detected from a field trial of transgenic plum cv. HoneySweet – Short Communication

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Abstract: A field trial with plum cv. HoneySweet was established in 2003 in an experimental plot near Crop Research Institute in Prague-Ruzyně, Czech Republic, on the basis of a permission issued by the Ministry of the Environment of the Czech Republic. In addition to the evaluation of resistance of plum cv. HoneySweet to *Plum pox virus*, the field trial was used to evaluate gene flow of the inserted transgene. Sampling of blackthorn and myrobalan trees outside the field test site occurred at distances ranging from 544 m to 845 m from the test site and showed no gene flow, testing both plants and seeds collected from blackthorns and myrobalans. Similarly, seeds from plums cv. Jojo growing directly at the field test place did not show any presence of the transgene after seven years of evaluation.

Keywords: *Prunus domestica* L. environment; *Plum pox virus*; resistance; transgene

Plum pox virus (PPV) is a dangerous viral pathogen of stone fruits, affecting most of their production areas worldwide (https://www.cabi.org/isc/datasheet/42203 Accessed April 25, 2018). Cv. HoneySweet is a promising plum (*Prunus domestica* L.) cultivar, showing gene silencing-based protection against PPV infection, mediated by an insertion of the PPV coat protein gene into the plum genome (Scorza *et al*. 1994, 2010).

It is approved for cultivation in the USA (Scorza et al. 2013a, 2016). In Europe, precautions are taken to minimise any possible risks which may be caused by introduction of foreign gene constructs into natural ecosystems by pollinating insects.

MATERIAL AND METHODS

In the Czech Republic, a field trial with cv. HoneySweet plum was established in 2003 in an experimental plot near Crop Research Institute in Prague-Ruzyně (Polák *et al*. 2017), on the basis of a permission issued by Ministry of the Environment of the Czech Republic. For the field trial scheme see Supplementary Figure S1 in EMS. The principle aim of the field trial is to evaluate the resistance of cv. HoneySweet to PPV (Polák *et al*. 2017). As part of this trial, gene flow monitoring is obligatory according to a Czech law (Act 78/2004 Coll.). Gene flow was monitored on four non-modified plums of Jojo cultivar, growing inside the field test plot only several meters from plums cv. HoneySweet. For long-distance gene flow monitoring, trees of *Prunus* species in the surrounding area were evaluated. Here we report the results of gene flow investigations.

To detect the gene flow to the plums (*P. domestica*) cv. Jojo planted in the field test plot, seeds of cv. Jojo were evaluated for the presence of the GUS transgene. Fruits were harvested and stones were taken from them, cracked open, the embryo removed and the epicotyl/hypocotyl, without cotyledons, was used for the test. The tested tissues were submerged into

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X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid cyclohexylammonium salt) solution prepared according to a standard protocol (Jefferson 1987). They were incubated at 37°C for 24 h, than the X-Gluc solution was removed, tissues were washed with absolute ethanol and visually examined. Seeds from non-transgenic plum trees were used as a negative control. Seeds from cv. HoneySweet were used as a positive control.

The field trial is located in an isolated area to minimize the risk of unwanted gene flow from modified plums. Only grasses and annual field crops like cereals are grown in surrounding fields. However, *Prunus* species are potentially cross compatible with *P. domestica* – blackthorn (*P. spinosa* L.) and myrobalan (*P. cerasifera* Ehrh.) – occurring in several locations around the field trial at distances from 544 m to 845 m. For the map of the area with locations of compatible *Prunus* species see Supplementary Figure S2 in EMS. The indicated locations of *Prunus* outside of the test site were searched for the presence of young, non-flowering plants that may have developed from seeds that could have been the result of unwanted natural gene flow mediated by pollinating insects since the field plot was established. All young trees were tested by X-Gluc test as described above, although some may have been root suckers from the older mother trees and would not have been the result of cross-pollination. Samples of leaves from non-transgenic plum trees were used as a negative control and leaves from cv. HoneySweet were used as a positive control. Also, seeds of mature flowering *Prunus* plants in the area were examined for the transgene presence, using the same protocol as for cv. Jojo. Seeds were collected in 2011–2017.

RESULTS AND DISCUSSION

During seven years of experiments, 306 young plants of blackthorns and myrobalans were tested and all were negative for the presence of the transgene (Table 1). Samples from cv. HoneySweet showed blue coloration after incubation with X-Gluc solution. We can conclude that no plant in a radius of 900 m from the field trial was of transgenic origin.

Table 1. Testing plants for the transgene presence (numbers of tested/positive plants)

Plum cultivar		Myrobalan	Blackthorn
HoneySweet	Jojo		
59/59	4/0	102/0	204/0

Table 2. Testing seeds for the transgene presence

Year	Plum cv. Jojo	Myrobalan	Blackthorn
2011	135/0	115/0	52/0
2012	121/0	96/0	208/0
2013	3/0	45/0	41/0
2014	303/0	85/0	42/0
2015	83/0	80/0	11/0
2016	264/0	152/0	82/0
2017	15/0	46/0	25/0

Numbers of tested/positive seeds; different numbers of tested seeds in individual years are due to late frosts and other factors causing losses of flowers and fruits, especially in plum cv. Jojo

Similarly, 2004 seeds from flowering myrobalans and blackthorns from the same locations as young plants, as well as all seeds from cv. Jojo from the trial site, were individually tested. Table 2 shows numbers of seeds evaluated in individual years. Low numbers of tested seeds in some years are due to a late frost causing fruit loss. However, all seeds from plums cv. Jojo, myrobalans, and blackthorns were negative for the transgene presence. Here we can conclude that no gene flow from cv. HoneySweet was detected.

The absence of gene flow outside the field plot is consistent with the report of Scorza *et al.* (2013b), who suggested, based on 11 years of *P. domestica* sampling, that beyond 400 m no gene flow would be expected. The gene flow in the present study was even more remotely possible since the sexual compatibility of *P. domestica* with myrobalan or blackthorn unaided by human intervention, as opposed to controlled breeding (Minev 2007), is extremely rare due to different ploidy levels (Nielson & Olrik 2001). *P. domestica* is hexaploid with $2n = 48$, myrobalan is diploid with $2n = 16$ and 24 and blackthorn tetraploid has $2n = 32$. Although in the case of blackthorn, natural hybrids with $2n = 16$, 24, 40, and 48 were described (OECD 2002). Moreover, pollinating insects, even wild species, tend to remain with a particular flowering species during the pollination process (Chittka *et al.* 1997; Hill *et al.* 1997; Stout *et al.* 1998) and will not cross-pollinate plums once they begin working myrobalans or blackthorns.

The lack of detectable gene flow even within the test field in the present study differs from the results of Scorza *et al.* (2013b) where a 4.9–39% rate of gene

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flow was detected within the test plot, depending upon the year. The difference in results may be explained in several ways. The Scorza *et al*. (2013b) field plot contained many different GE plum genotypes possessing the GUS transgene and was not limited to cv. HoneySweet. This plot also contained many cultivars of conventional, non-GE plums, therefore trees were flowering at different times and for a long period in spring and there was a great overlap of blooming between GE and non-GE plums. Also, plums with different cross-compatibilities were in the planting which allowed for more possibilities of successful cross-pollination. In the present study only plums cvs HoneySweet and Jojo were included in the test plot. While cross hybridisations conducted in the USA. between these two cultivars using cv. HoneySweet as the female parent indicated that they are cross compatible (R. Scorza, personal communication), there is not a complete overlap in blooming time. Although flowers of both cultivars were open simultaneously for several days in the current field test, giving insect pollinators a chance to cross-pollinate them. The lack of gene flow within the test plot based on 924 samples was unexpected and remains to be resolved. Pollination in the field trial is strictly dependent on wild pollinators, because no honeybee hives are allowed within a distance of 700 m from the field trial due to legislation restrictions for minimising the risk of long-distance distribution of transgenic pollen. Honeybee hives were included in the Scorza *et al*. (2013b) study and this may also account for the higher gene flow rate within the US field plot.

As a conclusion, our results, based on 306 leaf samples and 2004 seed samples collected over a 7-year period, show the absence of detectable gene flow.

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